American Academy of Allergy, Ninth Annual Meeting, Hotel Statler, Boston, Mass., February 26 to 28, 1953.

The Journal of Allergy

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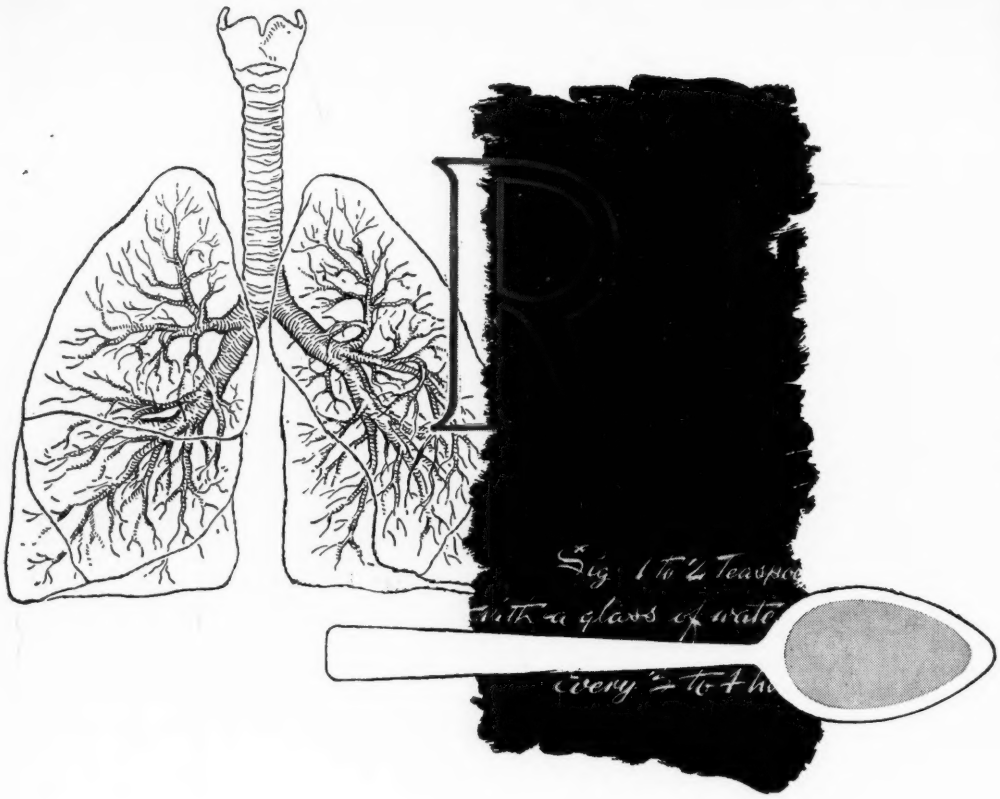
PUBLISHED BI-MONTHLY BY THE C. V. MOSBY CO. ST. LOUIS 3, MO.



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The Journal of Allergy

VOL. 24 JANUARY, 1953 NO. 1

Original Articles

FATAL AND NEAR-FATAL PENICILLIN ANAPHYLAXIS

THREE NEW CASES WITH A NOTE ON PREVENTION

SHEPPARD SIEGAL, M.D., ROGER W. STEINHARDT, M.D.,
AND ROBERT GERBER, M.D., NEW YORK, N. Y.

THAT penicillin may occasionally be a dangerous and even fatal drug should be known to the profession. In terms of direct toxicity the extraordinary antibiotic efficacy of this drug appears to have been matched by its innocuousness and as a result it is being used very freely. Allergic reactions have constituted the sole toxic manifestation attendant upon its administration.

For the most part the allergic reactions to penicillin have not been severe, being chiefly manifested by episodes of urticaria. However, more marked and serious reactions have been the subject of increasing by frequent reports. These have ranged from the complete serum sickness syndrome to instances of purpura with clear evidence of visceral improvement.[1, 2] More dangerous still are instances of exfoliative dermatitis, which may go on to fatal termination. In such cases autopsy may reveal an inflammatory reaction affecting the smaller veins and arteries.[3] Similar but even more extensive vascular lesions have been encountered at post-mortem examination in cases characterized by bullous purpura and fever.[4] Penicillin must therefore be added to the growing roster of chemotherapeutic agents which can initiate a widespread vascular reaction which may prove fatal even in this day of potent hormonal therapy.

The least known but most dangerous allergic reaction to penicillin is anaphylactic shock. Only a single fatal case of this kind has thus far been published, by Waldbott[5] in 1949. Nonfatal collapse reactions have been noted by several observers.[6, 7, 8, 9] In recent months Curphey[10] has presented two as yet unpublished fatal cases with complete autopsy data.

In the present paper are presented one case of death apparently due to penicillin hypersensitivity and two cases of unequivocal anaphylactic shock due to penicillin. It is certain that other similar cases have been unreported.[11] Even in the light of untold numbers of penicillin injections given without serious damage, these increasingly frequent observations of anaphylactic shock should serve as a warning.

From the Adult Allergy Clinic, The Mount Sinai Hospital.
Received for publication July 7, 1952.

CASE REPORTS

CASE 1.—S. C., a 67-year-old white woman, had bronchial asthma for several years. This was perennial and characterized by marked mucoid bronchorrhea. She had been studied completely from the allergic point of view elsewhere and had been found sensitive on skin tests to ragweed, plantain, feathers, kapok, and house dust. However, the major causative factor in her asthma was thought to be chronic sinus disease. Sinus x-rays showed thickened membrane. Her asthma was accentuated after upper respiratory infections. For this reason she had been treated with many antibiotics and had been given repeated injections of penicillin. To none of these had she shown any sign of intolerance. She was definitely allergic to aspirin which aggravated her asthma.

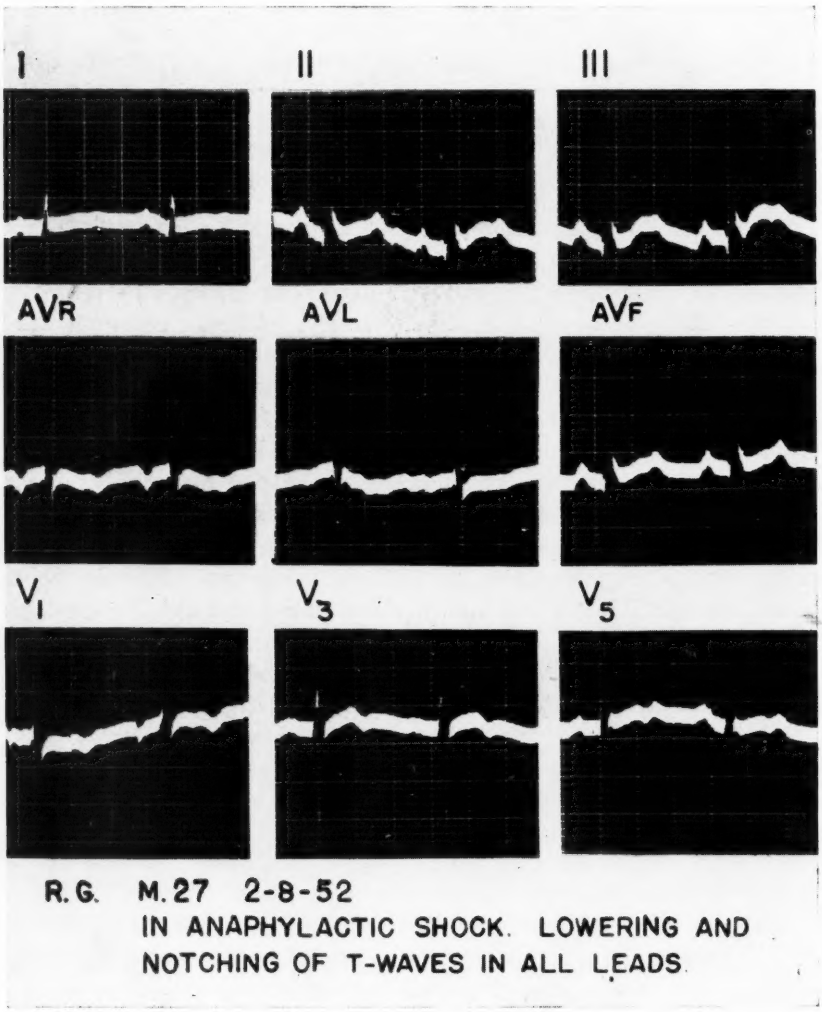

Fig. 1.—Case 2.

Apart from the asthma this patient was in good health. She had never had angina or any other evidence of heart disease. She had had no phlebitis, nor any indication of disease affecting the veins of her legs. Her blood pressure was 110/70. Electrocardiograms were

within normal limits. Complete blood count, blood glucose, nonprotein nitrogen, and cholesterol gave normal results.

On Oct. 25, 1951, she visited her physician while having rather severe asthma. Her temperature was 100° F. She was found to have acute follicular tonsillitis. She was therefore given an intramuscular injection of 300,000 units of aqueous procaine penicillin into the buttock. Within thirty seconds she complained of a strange taste in the mouth, tightness in the chest, and inability to breathe. Before epinephrine could be given she became extremely cyanotic and her labored respirations ceased. On auscultation there were no heart sounds. Intracardiac injection of epinephrine and artificial respiration were of no avail.

Acute toxicity tests carried out by the manufacturer with the penicillin from the vial used for the treatment of this patient were negative. An autopsy was not performed.

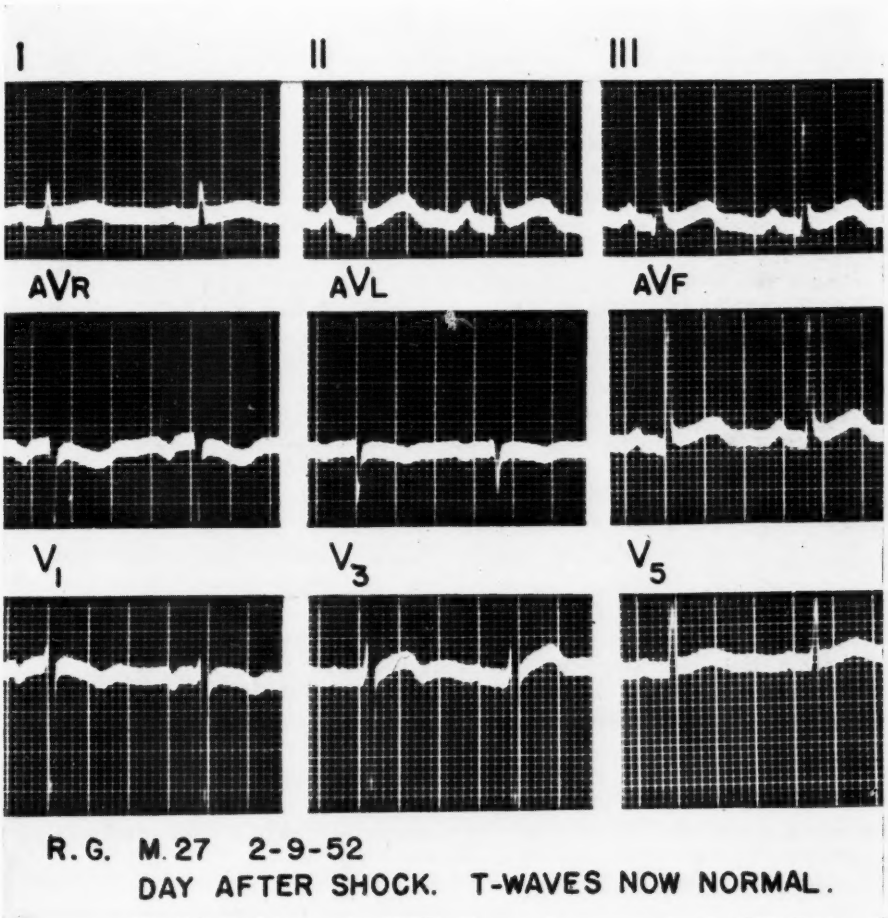

Fig. 2.—Case 2.

CASE 2.—R. G., a 27-year-old resident physician, awoke on Feb. 8, 1952, with sore throat which persisted throughout the day. Toward evening he stopped at the emergency ward for an injection of 300,000 units of aqueous procaine penicillin intramuscularly. Three minutes later while carrying on a telephone conversation his voice became husky and he had to clear his throat repeatedly. Then he felt lightheaded and this was followed abruptly by a severe sensation of pressure across the entire chest. He had an anesthetic feeling in

the back of his throat and a sense that his "larynx was about to close off." He was overcome by extreme weakness but was able to seek refuge in the neighboring electrocardiographic department. The sensation of chest constriction increased in intensity, his face became swollen and intensely pruritic, and he was covered with a cold sweat. He had severe nausea, repeated retching, and an urge to defecate. The acute phase of the attack lasted perhaps fifteen minutes.

Objectively, there was marked puffy swelling of the eyes, lips, and of the soft tissues of the neck. Slight cyanosis was present. A few fine râles with an occasional wheeze could be heard at the lung bases. His heart sounds were feeble, his pulse unobtainable, and blood pressure 50/30. In response to subcutaneous epinephrine and oral Pyribenzamine his edema subsided and his color improved.

An electrocardiogram taken during the shock reaction revealed lowering and notching of the T-waves in all leads, indicative of myocardial involvement (Fig. 1).

The following morning he was entirely comfortable. There remained a few crackling râles at the extreme lung bases. Chest x-ray showed no abnormality. A blood count showed no abnormality. Electrocardiographic examination now showed reversion of the T-waves to normal (Fig. 2).

His history as to previous exposure to penicillin was that he had received the drug by injection several times over a three-year period, without ill effects of any kind. The last previous penicillin injection was four months before the shocking dose.

His history as to allergy included some hay fever in the fall season and a tendency to dermographia present since childhood. His mother had light urticaria.

Scratch tests with aqueous crystalline penicillin G were carried out in late April, beginning with a dilution of 10 units per ml. Tests with increasing concentrations were negative until one was made with penicillin, 10,000 units per ml. After fifteen minutes there was a 2-3 plus reaction consisting of a 15 mm. wheal with moderate erythema of 20 mm. The control tests showed only a 2 to 3 mm. wheal and scarcely visible erythema. Scratch tests with aqueous procaine penicillin, 300,000 units per ml. and with 2 per cent procaine were negative. Passive transfer tests, carried out more than two months after the anaphylactic reaction, gave negative results on two recipients. For these tests penicillin G was used in 20,000 units per ml. concentration. Direct intracutaneous tests with ragweed and timothy pollens and animal danders gave strongly positive reactions.

CASE 3.—A. B., a 27-year-old resident physician, gave the history that he was first exposed to penicillin in 1947 and thereafter received repeated intramuscular injections of aqueous penicillin without untoward effect. In late 1949 he had his first reaction when within a few minutes following an injection of aqueous crystalline penicillin he developed an urticarial rash and edema of the hands and feet. This responded promptly to Pyribenzamine. In January, 1950, he was again given 50,000 units of penicillin intramuscularly and was almost immediately seized with marked dyspnea, likewise responding to an oral antihistamine.

Two months later, in March, 1950, he received an injection of 100,000 units of crystalline penicillin intramuscularly. Within thirty seconds he was overcome with a sense of collapse. There was a sudden urge to defecate. He was covered with a profuse sweat and he fell unconscious. His skin was a deep red, his breathing was labored, with an inspiratory crow and a cough productive of frothy sputum. Blood pressure and pulse were unobtainable.

Oxygen by inhalation, epinephrine subcutaneously, and Pyribenzamine by mouth induced relief of respiratory distress within ten minutes. Following recovery, nausea and extreme weakness persisted for several hours and there was residual dizziness for a brief additional period.

This patient had no clear-cut history of allergic disturbances previously. He was, however, aware that he sneezed readily on exposure to house dust and "caught cold" easily. This was usually promptly benefited by antihistamines. He had neither hay fever nor bronchial asthma and he recalled having urticaria briefly on one occasion only many years ago. There had been no other evidence of drug allergy. His family history was positive for

allergy in that one sister has marked summer and fall hay fever and another had a near-fatal reaction to diphtheria antitoxin. His mother had penicillin urticaria.

No attempt was made to test this patient directly with penicillin. Passive transfer tests, carried out two years after the anaphylactic shock, gave negative reactions to crystalline penicillin G, 20,000 units per ml. Direct and passive transfer tests with pollen extracts were negative.

DISCUSSION

Only a single case of fatal penicillin anaphylaxis has been published thus far. In 1949, Waldbott[5] reported upon a 39-year-old female patient with asthma whose fatal reaction followed an intramuscular injection of 50,000 units of aqueous crystalline penicillin. Within minutes she complained of a strange taste in the mouth, tightness in the throat, and generalized itching. There was facial flush, extreme cyanosis, collapse and death. She had been treated with penicillin previously without any untoward reaction whatsoever.

In March, 1952, Curphey[10] presented a report on two cases of fatal anaphylaxis following penicillin in two patients with bronchial asthma. One patient died within five minutes after receiving aqueous procaine penicillin intramuscularly and at autopsy the chief finding was that of voluminously distended lungs with marked dilatation of the alveoli. These findings were consistent with the picture of "guinea pig anaphylaxis," as has been reported previously in other instances of fatal human anaphylactic shock.[12] His second patient died within ten minutes after an injection of penicillin and streptomycin combined. At autopsy the most noticeable finding was that of extensive mucoid exudate in the smaller bronchi and alveoli. In neither case was there found any other cause of death.

If we add Case 1 of the present report, it is striking that all four fatal cases of penicillin anaphylaxis were in patients with bronchial asthma. Of great practical import too is the fact that one of them had exhibited only questionable indication of previous penicillin allergy, and in the other three there was not the slightest hint of possible fatal anaphylaxis.

Since autopsy was not obtained in two of the cases it cannot be stated beyond any doubt that some other cause of sudden death was not present. Nonetheless, the fatality directly after injection, the accompanying asthma, and the manner of exitus all point strongly to true anaphylactic death.

Although other antigenic components such as procaine or streptomycin cannot be completely excluded as possible causative factors in two of these cases, the penicillin itself appears to be the most probable cause. This conclusion is considerably strengthened by the observations made in the nonfatal cases to be discussed.

Four nonfatal anaphylactic reactions to penicillin have been described previously. Burleson's[7] patient reacted to aqueous crystalline penicillin, mixed with procaine, with sudden dyspnea, generalized pruritus, and collapse. An immediate intracutaneous test with penicillin gave a positive reaction, whereas that to procaine was negative. Everett's[8] patient twice underwent severe shock inasmuch as penicillin was not at first recognized to be the causative agent. The first reaction followed the instillation of 30,000 units of aqueous crystalline

penicillin G solution into the maxillary antrum. Facial flush, dyspnea, wheeze, collapse, and unconsciousness supervened. Three months later intramuscular procaine penicillin in peanut oil induced an identical reaction. This patient had received penicillin by instillation into the antrum and by injection repeatedly in the previous four years without incident. He was not asthmatic and apparently not atopic.

The patient reported by Irwin and associates[9] was, as in Case 2, and Case 3 of the present report, a physician. Again parallel with Case 3, he failed to heed a previous immediate reaction to penicillin. After a three-week interval he was given procaine penicillin in oil and in thirty seconds felt a generalized sensation of heat, with scrotal itching and dyspnea, and went into collapse. He was subsequently scratch-tested with crystalline penicillin, 1,000 units per ml. in aqueous solution, and reacted with a huge zone of whealing and erythema. A similar test with procaine was negative. Passive transfer tests were positive in one recipient, negative in two others.

All six cases of nonfatal anaphylactic shock, including the two here first reported, were almost certainly due to penicillin itself, and not to accompanying procaine or vehicle. Two of these patients had experienced previous immediate reactions to penicillin which should have served as a warning of possible anaphylactic sensitivity. The patient described by O'Donovan and Klorfajn[6] had penicillin contact dermatitis, and reacted with collapse to an injection of penicillin.

In Burleson's[7] case there was only questionable evidence of previous penicillin allergy. In the remaining two cases there was nothing whatsoever to serve as a warning that a state of anaphylactic sensitivity to penicillin had been induced.

PREVENTION

Despite the apparent rarity of dangerous reactions to penicillin it is our contention that these experiences call for far greater circumspection in the use of penicillin than is the current practice. It appears probable that penicillin anaphylaxis is on the increase as more and more persons attain a high level of sensitization through repeated exposure to the drug. It would therefore seem the wiser course to avoid needless hypersensitization of patients through indiscriminate use of this antibiotic.

1. For many minor respiratory infections which are mostly viral in nature, no specific antibiotic is available and chemotherapy should be avoided altogether. When penicillin is distinctly the drug of choice oral preparations should be used more frequently in mild to moderate infections. It has been emphasized increasingly that for such conditions one or two 200,000 unit tablets three times daily often comprises effective therapy.[13, 14] While sensitization may occur as well by the oral route, the occurrence of anaphylactic shock should thus be reduced and anaphylactic death possibly prevented altogether. Immediate drug fatalities of this type almost never occur after oral administration but are not too rare after parenteral injection.[15] It is for this reason that Feinberg[16] stated: "... it is incumbent upon the conscientious physician to observe the strict rule that nothing that is effective by mouth should be given by injection."

2. Before administering or prescribing penicillin a careful history should be elicited as to its previous administration and the occurrence of any reaction. An accurate history should also be taken as to both personal and familial allergy, particularly as to bronchial asthma.

Anaphylactic shock to penicillin need not be feared in patients who have never been exposed to the drug previously. Some confusion may exist on this score because some individuals have latent penicillin sensitivity presumably based upon pre-existing dermatomycosis. The initial exposure to penicillin in such patients may elicit a cutaneous reaction of the "id-like" erythematovesicular type, but has not been known to cause anaphylactic shock.

Anaphylactic shock has thus far been observed after single injections of penicillin in patients intermittently treated and not during a course of continuous therapy. This appears analogous to the common experience with animal anaphylaxis where adequate spacing of antigen injections greatly enhances the likelihood of inducing shock.

In evaluating the history of previous penicillin allergy the physician should distinguish sharply between the common *delayed* reactions on the one hand and the uncommon *accelerated or immediate reactions* on the other. The delayed reactions occur typically after an incubation period of seven to twenty-eight days, are often mild and characterized by some or all of the elements of the serum sickness syndrome. For the most part these are associated with only a transient period of sensitization and such patients often tolerate a subsequent course of penicillin without difficulty.[17, 18] In sharp contrast, the accelerated and immediate reactions come on within a matter of minutes or hours, or at most a day or two after penicillin. Immediate reactions tend to be more violent, but even though such a reaction may be mild, its timing should serve as a warning that penicillin sensitivity is present and anaphylactic shock a distinct probability. A possible exception to this rule is the "id-like" erythematovesicular reaction which may appear within twenty-four hours after penicillin treatment. The morphology of this eruption and its typical hands-feet-groin distribution should readily differentiate it, a matter of practical importance, as such sensitivity is apparently not indicative of an anaphylactic danger.

Apart from the immediate and accelerated allergic reactions to penicillin certain other forms of penicillin allergy are more likely to be associated with severe degrees of sensitivity. Thus a history of exfoliative dermatitis or a purpuric reaction indicates the need for special care. Patients with a contact allergy to penicillin, as seen particularly in doctors and nurses, should not be treated with the drug as they may react dangerously. Moreover, it should be noted that regardless of the character of reaction to penicillin, patients who have had more than a single episode of penicillin allergy should be treated with caution. Last, it must be understood that the absence of any previous allergic reaction to the drug by no means precludes the possibility of anaphylactic sensitization. Indeed, several of the patients here reported or referred to had had no previous untoward reaction to the drug.

3. Skin tests with penicillin may be helpful in the prevention of anaphylaxis. Experience is thus far too limited to present a definitive evaluation but the

observations made in Case 2 of the present report, in the case reported by Irwin and associates,[9] and in Burleson's[7] case suggest that anaphylactic sensitivity to penicillin may in some cases be associated with a positive immediate skin test. This is in contrast with most forms of drug allergy in which negative skin tests are the rule.

Penicillin skin testing has thus far been done with aqueous solution of penicillin G, and it is this solution which should be employed for the purpose of detecting the anaphylactic state. There is no published experience with tests made with procaine penicillin, which may release penicillin too slowly to induce an immediate reaction on scratch test (Case 2). Penicillin G solution has the disadvantage of being unstable at room temperature and even under refrigeration loses both potency and antigenicity within a week. However, 100,000 unit vials of powdered, dry crystalline penicillin G can be kept at hand in the doctor's bag, in offices, and in hospitals specifically for use in skin testing.

For routine use a scratch test is safest as the intracutaneous test may rarely induce shock.[19] Crystalline penicillin G, 10,000 units per ml. in distilled water, should be used and the reaction read at twenty minutes. A positive test may be missed if the reading is done too soon. The positive reaction consists in a definite wheal and flare. Such a test would serve as a crude screening procedure of particular value for: (1) patients who have had repeated previous administration of penicillin; (2) those who have a history of a previous reaction to penicillin, especially if recent or if unusually severe; (3) for atopic individuals and particularly for the asthmatic patient. When convenient, immediate intracutaneous tests with aqueous penicillin may also be carried out if the scratch test has proved negative.

It cannot be emphasized too strongly, however, that negative skin tests do not rule out penicillin allergy or anaphylaxis. It is therefore safer to avoid penicillin therapy in patients with a history of an immediate or accelerated reaction to penicillin despite negative skin reactions. When there has been a previous delayed reaction to penicillin of the usual type it is often wise to administer preliminary smaller doses of penicillin, perhaps[18] 5,000 to 10,000 units, before giving the full therapeutic amount.

In instances in which the overriding consideration is the urgent need for penicillin despite a strong suspicion of anaphylactic sensitivity, a series of trial injections of penicillin in ascending dosage might be indicated. The initial dose would be from 20 to 50 units given subcutaneously into the upper extremity, injections being repeated at intervals of one-half to one hour, with the hope that eventually a dose of therapeutic value would be attained. Whenever the risk of anaphylactic reaction is to be taken it need hardly be emphasized that a syringe filled with aqueous solution of epinephrine must be at hand as well as an injectable solution of antihistamine and a tourniquet. Such a procedure is best carried out in the hospital.

The delayed penicillin intracutaneous test, performed with 0.1 ml. of aqueous solution, 10,000 to 20,000 units per ml., and read at twenty-four to forty-eight hours, is not discussed here. Although much the most commonly positive of all penicillin skin tests, it has little place in the prevention of

anaphylactic reactions. Its area of usefulness and its limitations have been described previously.[18]

4. The technique of penicillin injection deserves critical appraisal in the effort to avoid anaphylaxis. Injections, especially the first of a series, should be given into the outer aspect of the upper arm rather than into buttock or deltoid, so that if necessary a tourniquet, ready at hand, may be applied proximal to the site of injection. Accidental intravenous injection should be avoided by the routine measure of tugging on the plunger and observing whether there is blood in the syringe before actual administration.

In discussing the prevention of anaphylactic shock from other substances, Waldbott[20] suggested that the first few drops of solution be injected, followed by a forty-five second pause before the remainder of the dose is given. This seems sensible in that the potentially fatal reactions may become evident within this period (Case 3). Epinephrine should be directly at hand whenever a penicillin injection is to be given.

The precautions just outlined are intended particularly for the care of asthmatic and atopic persons and for any patient who gives a history of penicillin allergy. Nonetheless, all may benefit from greater caution in the use of the antibiotic.

SUMMARY

1. One death and two cases of nonfatal anaphylactic shock due to penicillin are described.

2. The fatality occurred in a patient with bronchial asthma. Three other known deaths attributable to penicillin anaphylaxis have also occurred in asthmatics.

3. Two of these patients, including the one with a fatal reaction, had received penicillin many times before without indication of allergy. The third patient responded previously with immediate reactions to penicillin which went unheeded as a warning of anaphylaxis.

4. A direct scratch test with aqueous crystalline penicillin was positive in one case, whereas similar tests were negative with aqueous procaine penicillin and 2 per cent procaine. Passive transfer tests were negative.

5. Detailed precautions are suggested toward the prevention of penicillin anaphylaxis, particularly for asthmatic and penicillin-allergic persons. The avoidance of the indiscriminate use of penicillin, particularly by injection, is emphasized. The value of a systematic inquiry as to previous penicillin treatment and reactions as well as of the general allergic status is stressed. Certain refinements in the method of penicillin injection are suggested. The technique and interpretation of preliminary penicillin skin tests are discussed.

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30 EAST 60TH ST.

THE RELATIONSHIP OF EXPERIMENTALLY PRODUCED ASTHMATIC ATTACKS TO CERTAIN ACUTE LIFE STRESSES

DANIEL H. FUNKENSTEIN, M.D.
BOSTON, MASS.

THE relationship of the occurrence of asthmatic attacks to real-life stress-inducing situations has been reported by many investigators who made the interpretation that the asthma represented a reaction to the life situation.[13-15] Wolff[15] and others have used this method intensively for studying the relevance of emotional factors to the onset of exacerbations of other psychosomatic illnesses such as ulcerative colitis,[9] migraine,[12] and hay fever.[11]

Elsewhere we reported[5] a number of asthmatic patients whose asthmatic attacks ceased when they became psychotic. When their psychoses cleared, the clinical asthmatic attacks returned. Paralleling the psychologic changes, it was further shown that the ability of Mecholyl (acetyl-beta-methyl choline), a drug which precipitates asthma only in asthmatic patients,[2] to precipitate asthma changed. During the psychotic asthma-free period, Mecholyl precipitated only short mild asthmatic attacks as contrasted with the severe sustained asthmatic attacks precipitated by the same drug in these patients in their psychotic-free periods.

Previous work in this laboratory,[4] in which the cardiovascular system was used as a measure of the response to stress, showed two main mechanisms associated with acutely elevated blood pressures in healthy normotensives. One pattern was similar to that obtained after the injection of synthetic epinephrine (increased pulse rate, increased cardiac output, and decreased peripheral resistance), and the other pattern was similar to that obtained after the injection of nor-epinephrine (unchanged, slightly increased or decreased pulse, decreased cardiac output and increased peripheral resistance). Hickam, Cargill, and Golden[10] and Goldenberg and associates[8] reported similar findings.

In the study reported in this paper, the cardiovascular responses of asthmatic patients to psychologic stresses were correlated with the severity and duration of asthmatic attacks experimentally produced by the injection of the drug Mecholyl.

METHOD

Subjects.—Eight male college students, who were known to have frequent asthmatic attacks but who had been free of such an attack during the preceding four weeks, were used as subjects. The only other criterion of selection was willingness to take part in the experiments for a fee. Their average age was 21.7, ranging from 19 years to 27 years.

From the Department of Psychiatry, Harvard Medical School, and the Boston Psychopathic Hospital.

The research reported in this article was made possible (in part) by a grant from the Supreme Council, 33rd Degree, Scottish Rite, Northern Masonic Jurisdiction, U.S.A., through the National Association for Mental Health.

Mecholyl was Furnished by Merck and Company.

Read at Regional Research Conference, American Psychiatric Association, Boston, Mass., Feb. 28, 1952.

Received for publication, June 11, 1952.

Seven students were studied during a real-life stress-inducing situation and again during a nonstressful situation. One of the students was studied during 2 life stress-inducing situations and 2 nonstressful situations. The life stress-inducing situation for the 6 college students was the night prior to a final examination, and for the 2 medical students the night of internship announcements. One of the students was also studied during an additional real-life stress-inducing situation, namely, a crisis in his family's interpersonal relations.

Measures.—The following measures were used. *Stress:* During the psychological stress-inducing situations the criteria used to determine that the students actually were under stress were their subjective reports that they felt tense, anxious, and/or angry. They appeared emotionally disturbed. Conversely, during the nonstressful situations, they all stated that they felt relaxed and that nothing unduly stressful had occurred in their lives during the previous twenty-four hours. They appeared calm and relaxed at that time. *Asthmatic Attacks:* The reactions after Mecholyl in terms of asthmatic attacks were scored as follows.

"No asthmatic attack precipitated"—Here the subjects complained of no subjective asthma or dyspnea and there was no objective evidence of an attack of asthma.

"Mild asthmatic attack precipitated"—Here the subjects complained of asthma and some dyspnea and there was objective evidence of slight dyspnea but the subjects did not have to sit up to ease their breathing. The attacks terminated within 7 minutes.

"Severe asthmatic attack precipitated"—Here the subjects complained of asthma and severe dyspnea. There was objective evidence of dyspnea of such magnitude that the subjects had to sit up to ease their breathing. The attacks in all of these instances lasted over 20 minutes, and 2 of them had to be terminated by giving epinephrine.

Cardiovascular Measures.—The blood pressure was obtained by the cuff and auscultatory method. The Sanborn modification of the Dock cardioballistograph was used.[3] The cardioballistograph is a crude instrument discovered empirically but an instrument which gives valuable experimental data.[3] The values obtained are not the true values but an approximation of them. With this fact in mind, and due to the difficulties of standardizing the machine, the figures we have used are the raw data. In calculating the data, the IJ wave was expressed in terms of the length of this segment in mm. divided by the time of the wave in seconds. No effort was made to convert this figure into grams of force and thus into cubic centimeters of volume. The pulse was obtained from the cardioballistographic tracing, and using the IJ wave as indicated above, the "stroke volume" and "cardiac output" were calculated by a modification of Nickerson's formula:[14] $SV = \sqrt{\frac{P}{T}} \times IJ$.

The "over-all peripheral resistance" was then calculated from the other data by the method of Aperia:[1] $TPR = \frac{P}{CO}$. The terms "calculated stroke

volume," "calculated cardiac output," and "calculated peripheral resistance" were used.

These values permit the comparison of the same individual's cardioballistographic record at one time with his record at another, and thus changes within the same individual on various occasions and under different conditions can be determined. This method does not allow for a comparison of absolute values between individuals. In the experiments reported in this paper, the changes in each individual between the stressful and the nonstressful situation were compared. These differences were converted into percentage deltas in order to get a common score with which to compare the magnitude of changes in the different subjects.

Procedure.—The experimental procedures carried out on each subject during the stress-inducing situation and during the nonstressful situation were identical. The time interval between the two situations was between 7 and 14 days.

After inquiry was made about the subject's subjective feelings, he was allowed to lie supine on the cardioballistograph table for 30 minutes. At the end of that time, the blood pressure was taken and a cardioballistographic tracing was made. He was then given 10 mg. of Mecholyl intramuscularly and both the subjective and objective evidences of asthma, including both severity and duration, were noted. The chest was auscultated for sonorous and sibilant râles.

RESULTS

First the cardiovascular data were analyzed. There was a higher blood pressure during the stressful situation as compared with the nonstressful situation in all of the subjects. The average difference was 24.7 mm. Hg systolic (21.6 per cent), ranging from 12 mm. Hg (9 per cent) to 40 mm. Hg (38 per cent). The average difference in the diastolic pressure was 10.2 mm. Hg (13.7 per cent rise), ranging from –6 mm. Hg (–8 per cent fall) to 30 mm. Hg (38 per cent rise).

The cardiovascular patterns were then analyzed according to the previously stated variables for epinephrine and nor-epinephrine. Six of the patterns were of the epinephrine type (increased pulse, increased "calculated cardiac output" and decreased "calculated peripheral resistance"), and three patterns were of the nor-epinephrine type (unchanged, slightly increased or decreased pulse, decreased "calculated cardiac output" and increased "calculated peripheral resistance"). Table I.

The data in regard to the asthmatic attacks were then classified for both stressful and nonstressful situations into the type of asthmatic attack produced. During the stressful situations, the reaction to Mecholyl was scored as producing a "mild asthmatic attack precipitated" on 6 occasions and "no asthmatic attack precipitated" on three occasions. During the nonstressful situation, the reaction after Mecholyl was scored as "severe asthmatic attack precipitated" in all 9 instances (Table I).

TABLE I. CARDIOVASCULAR CHANGES AND THE TYPES OF ASTHMA ATTACKS PRECIPITATED BY THE

		STRESS								NONSTRESSFUL		
		TYPE OF ASTHMA PRECIPITATED BY MECHOLYL			CARDIOVASCULAR					TYPE OF ASTHMA PRECIPITATED BY MECHOLYL		
SUBJECT	AGE	NONE	MILD	SEVERE	B.P.	PULSE	"CALCULATED STROKE VOLUME"	"CALCULATED CARDIAC OUTPUT"	"CALCULATED PERIPHERAL RESISTANCE"	NONE	MILD	SEVERE
1	26		*		150/82	72	750	54	2.15			*
2	27	*			126/74	84	954	80	1.25			*
3	23	*			130/78	78	891	69.6	1.49			*
4	19		*		126/80	71	1065	75.6	1.36			*
5	20	*			130/100	65	768	50.0	2.30			*
6	20		*		130/110	63	577	36.4	3.30			*
7	19		*		148/108	75	1078	80.6	1.59			*
8, *a*	20		*		170/70	95	1590	151	.795			*
8, *b*	20		*		170/90	97	1940	188	.692			*

DISCUSSION

The results clearly showed that during psychologic stress, Mecholyl usually produced milder and shorter asthmatic attacks in asthmatic patients, than when this same drug was given to these same individuals during a nonstressful situation. In three of the subjects no asthmatic attacks were precipitated during the stressful situation as compared with severe asthmatic attacks precipitated in these same individuals during nonstressful conditions.

In an analysis of the cardiovascular patterns, three of the subjects showed patterns during stress similar to those which can be obtained by the injection of nor-epinephrine, and six of the subjects showed patterns during stress similar to those which can be obtained by the injection of synthetic epinephrine. On the basis of this evidence the interpretation is made that excessive secretion of nor-epinephrine-like or epinephrine-like substances during stress could account for the absence or mildness of the asthmatic attacks precipitated by Mecholyl at that time.

Grace, Wolf, and Wolff[9] reported that the physiologic action of drugs on the autonomic nervous system varied with the emotional states of patients. The work reported in this paper was further confirmatory evidence of their observations. It is evident that the effects of Mecholyl depended largely on the emotional states of the patients and its physiologic effects shifted with changes in the patients' emotional states. Other work of ours has made use of this observation in developing a test related to the prognosis of patients treated with electric shock therapy.[6]

Previously we reported that in psychoses developing in asthmatic patients, evidence of altered activity of the sympathetic nervous system could account for the failure of Mecholyl to produce asthmatic attacks during the psychoses.[5] In the cases reported in this study, evidence was found for altered activity of the sympathetic nervous system during psychologic stress in asthmatic patients, which could account for the failure of Mecholyl to produce severe asthmatic attacks at that time. When the psychoses were over in the cases reported previously, or when the acute psychologic stress terminated in the

INJECTION OF MECHOLYL FOR EACH OF THE SUBJECTS IN THE STRESSFUL AND NONSTRESSFUL SITUATIONS

SITUATION					PER CENT DELTA NONSTRESS AND STRESS					
CARDIOVASCULAR										
B.P.	PULSE	STROKE VOLUME	CARDIAC OUTPUT	PERIPHERAL RESISTANCE	B.P.	PULSE	"CALCULATED STROKE VOLUME"	"CALCULATED CARDIAC OUTPUT"	"CALCULATED PERIPHERAL RESISTANCE"	TYPE OF PATTERN
110/62	43	581	24.7	3.48	36/32	67	29	84	−38	Epinephrine-like
110/68	71	515	36.4	2.44	15/9	18	85	119	−49	Epinephrine-like
94/68	57	715	40.8	1.98	38/15	37	29	71	−25	Epinephrine-like
104/68	72	1209	87.0	0.99	21/17.6	− 1.3	−11.9	−13.1	+37.3	Nor-epinephrine-like
118/98	72	1220	88.0	1.23	101/2	− 9.7	−37.0	−43.1	+86.9	Nor-epinephrine-like
112/80	53	790	41.8	2.29	16/37.5	+18.8	−26.9	−12.9	+44.1	Nor-epinephrine-like
136/94	63	805	50.7	2.26	9/19	19	34	59	−30	Epinephrine-like
130/76	71	1300	92.4	1.12	31/−8	34	22	63	−29	Epinephrine-like
143/90	69	1540	106	1.05	19/0	41	26	77	−34	Epinephrine-like

nonpsychotic patients reported in this paper, Mecholyl again precipitated severe asthmatic attacks. This evidence suggested that during their psychoses, the patients were under constant stress, the stimulus of which was internal, and that the altered autonomic physiology represented part of the stressful reaction.

The question arises as to why these asthmatic patients did not react to the stressful life situations with the production of asthmatic attacks, or with an increased sensitivity to Mecholyl with resulting increase in the severity of the asthmatic attacks precipitated by the drug. In view of the numerous reports of the relation of life stresses to asthma, these experiments were carried out with considerable trepidation, as our hypothesis was that during the stress the asthmatic attacks produced by Mecholyl would be more severe than those precipitated in these same subjects during the nonstressful situation. As indicated previously, our results were entirely opposite to the hypothesis.

There is no answer from the data concerning the above problem, but the following possibilities deserve consideration.

1. Asthma is multidetermined, and in these cases other factors such as allergy were not operative at the time of the experiments.

2. Our cases were too few in number to allow for definitive answers.

3. The cases reported here were subjected to acute stress, and it may be that stress must be prolonged prior to the onset of asthmatic attacks.

4. It may be that the psychological situation must carry special significance for the individual for the development of asthma, and our situations did not have this meaning to our subjects.

5. Stresses which produce asthmatic attacks stem from unconscious rather than conscious conflicts.

6. Ability to verbalize emotions may be an important factor and the patients in this study had no difficulty in verbalizing their feelings. Others have reported that inability to express emotions may be an important factor in producing asthmatic attacks.

7. Unknown factors.

We have no explanation as to which of the above possibilities are the correct solution of the problem, but this work does emphasize the difficulty of evaluating life stresses in relation to the occurrence of asthmatic attacks when only the history is used.

One of the cases which was very interesting is included as an addendum. This subject (No. 8 in Table I) was seen during 2 stress-inducing situations and 2 nonstressful situations. One stress-inducing situation was the night before an important final examination, and the other was after an intense argument with his father, who told him that either his wife should go to work immediately or he would no longer support him in college. The subject was resentful, anxious, and emotionally disturbed at this time. Tests were run on him within an hour of the heated argument with his father.

In these seemingly dissimilar stress-inducing situations, the subject showed an elevated blood pressure of the epinephrine-like type on both occasions. Only a mild asthmatic attack could be precipitated by Mecholyl during the stressful situations, as contrasted with the severe asthmatic attacks precipitated in this subject during the 2 nonstressful situations.

SUMMARY AND CONCLUSIONS

1. Seven asthmatic subjects were studied during a real-life stress-inducing situation, and one asthmatic patient was studied during two real-life stress-inducing situations. All eight subjects were each studied during at least one nonstressful situation.

2. Mecholyl, a drug which precipitates asthma only in asthmatic patients, produced severe asthmatic attacks in all eight subjects during the nonstressful situations. In contrast to this, during the stressful situations, this same drug produced no asthma or only mild asthmatic attacks in these same individuals.

3. Cardioballistographic studies made during the stress-inducing situations and the nonstressful situations in these individuals showed patterns during stress on 3 occasions similar to those which can be obtained by the injection of nor-epinephrine, and on 6 occasions similar to those which can be obtained by the injection of synthetic epinephrine.

4. The interpretation was made on the basis of the above data that excessive secretion of an epinephrine-like or nor-epinephrine-like substance during psychologic stress could account for the failure of Mecholyl to produce severe asthmatic attacks at that time.

The author gratefully acknowledges the technical assistance of Lydia W. Meade, R.N., and Harry C. Solomon, M.D.

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THE HISTAMINE CONTENT OF ALLERGIC AND NONALLERGIC HUMAN NASAL MUCOUS MEMBRANE WITH SIMULTANEOUS OBSERVATIONS ON THE EOSINOPHILS

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THE concept of histamine release from tissue as a result of the combination of antigen with antibody has found support in numerous studies on animal anaphylaxis. Both an increase in the histamine content of blood in the intact animal, and of perfusate from isolated tissue have been demonstrated following administration of the specific antigen. As might be expected, a corresponding decrease in the histamine content of the perfused tissue can also be readily demonstrated.[1]

In attempts to relate the symptomatology of allergy in man to a release of pre-existing histamine, equivocal as well as completely negative results have been the rule. Thus, using cardiac catheterization as a means of obtaining blood both before its entry into and just after its exit from the lungs, no significant alteration of the blood histamine content could be demonstrated following the induction of clinical asthma.[2,3] One striking exception to this is the clearly demonstrable release of histamine in the condition of cold hypersensitivity.[24]

Because of the obvious limitations and difficulties which arise during investigative studies on human tissue, only a few pertaining observations have appeared in the literature. Buhrmester and Wenner[2] reported that on the basis of moist weight nasal mucous polyps and normal nasal mucous membrane showed almost identical histamine values. On the basis of a calculated dry weight, however, the polypoidal tissue contained more histamine than the normal. Their normal tissue was obtained from cadavers. In recent work carried out by Rose, Entin, and Baxter[3] on the histamine content of various human tissues, several high values (101 and 140 γ/gram) were obtained on antral mucous membrane from allergic individuals. The exact significance of such findings is not clear.

The scanty evidence available to date suggests that there is an increase in the histamine content of human nasal mucous membrane during an allergic reaction. The following investigation was undertaken in an attempt to assess what actually happens to the histamine under such circumstances. Simultaneous observations were made, whenever possible, on the eosinophils in the peripheral blood, nasal tissue, and nasal secretion. The findings were correlated with the degree of the allergic reaction present.

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Aided by a grant from the National Research Council of Canada. Part of this work was supported by the CIBA Company, Montreal.

Received for publication Nov. 17, 1952.

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I. SELECTION OF CASES

A. *Allergic:* The allergic patients reported were cases of nasal mucous polyps. Primarily due to the work of Kern and Schenck[4, 5, 6] it is generally recognized today that nasal mucous polyps have an allergic basis.[7, 8, 9, 10] They occur particularly in those types of respiratory allergy that are protracted and perennial. In such cases the persistent allergic edema of the tunica propria in certain specific areas plus the effects of gravity leads to a prolapse of the nasal mucosa with subsequent polyp formation.[10]

There still remains appreciable support in the literature for the view that nasal mucous polyps are inflammatory in origin.[11, 12] The inflammatory school feels that polyps arise because of basic vascular changes in the nasal mucosa induced by chronic or repeated attacks of infection. Many nasal mucous polyps, however, occur without any preceding nasal infection and the general view is held today that allergy is the basic etiological factor in the vast majority of chronic nasal and sinus infections.[13, 14] On the basis of these concepts cases of nasal mucous polyps were used in this study.

The patients were investigated completely from an allergic point of view and the various findings correlated with the degree of the allergic reaction present at the time. The degree of the allergic reaction in the nasal mucosa of patients with nasal allergy, with or without mucous polyps, is variable. At periods, depending upon several factors, the allergic reaction may be at a minimum or even quiescent while at other times it is quite severe.

The degree of nasal signs and symptoms varies directly with the degree of the allergic reaction occurring in the nose. If the allergic reaction is severe, the nasal signs and symptoms are severe, and vice versa. Critical observations of the degree of nasal signs and symptoms can, therefore, be used as an indication of the degree of the allergic reaction occurring in the nose.

Although this is essentially true in cases with nasal mucous polyps, there is here the added factor of a mechanical blockage of the nasal passages by the polypoidal masses. In certain cases, although the allergic reaction may be quiescent, the patients complain of marked nasal blockage which is purely on a mechanical basis. One must remember this in assessing the symptoms of patients with nasal polyps and particular attention should be paid to the condition of the nasal mucous membrane as such. In this study the degree of the allergic reaction present at the time the nasal polyps were removed was recorded and efforts made to remove tissue during various phases of the disease process.

B. *Nonallergic:* As no acceptable series of histamine determinations on normal human nasal mucous membrane could be found in the literature it was necessary to establish control values. Through the cooperation of the Department of Otolaryngology, The Royal Victoria Hospital, facilities to obtain sufficient normal nasal mucous membrane for histamine determinations were provided. The "normal" patients were cases either undergoing tonsillectomy, adenoidectomy, tonsillectomy and adenoidectomy, or submucous resection—designated in charts as T, A, T&A, and SMR, respectively.

At the time of operation these patients were all asymptomatic as far as their local condition was concerned and all cases selected were free from any obvious complicating nasal condition. The patients were carefully screened for any personal or family history of allergy. Blood and nasal smears were examined for eosinophils and if there was the slightest indication that any "allergic" tendencies existed they were excluded from the normal series. They were therefore considered as normal individuals undergoing elective surgery.

In order to determine if the histamine content of nasal mucous membrane varied with different age periods or sex, the patients were divided into groups according to age and sex. The age groups were as follows:

Group 1. From birth to fourteen years of age—the child or prepuberty group.

Group 2. From 15 to 44 years of age—the adult group.

Group 3. From 45 years of age and onward—the older adult and postmenopausal group.

The ages of 14 and 44 years were selected as the dividing points of the groups because they are the broad average ages for puberty and the menopause to occur, respectively. Around these ages there are adjustments occurring in the endocrine system of the body and, associated with this, changes in tissue histamine could conceivably take place.

II. METHODS

A. *Methods of obtaining nasal mucous polyps:* The nasal mucous polyps were all obtained by essentially the same method. They were removed, under local anesthesia, with a nasal snare. The biopsy specimens were placed between layers of gauze moistened with normal saline. They were then transported as quickly as possible to the laboratory where the histamine determinations were performed. In order to study the effect of local anesthesia (cocaine and epinephrine) on the histamine content of polyps, patients with multiple polyps were used. First a polyp was removed without anesthesia, and this was immediately followed by removal of another under anesthesia. No significant change was found to occur in the polyp histamine content under these varied conditions.

B. *Method of obtaining normal nasal mucous membrane:* All the normal nasal mucous membrane was obtained by essentially the same method. At the termination of the scheduled operative procedure, while the patient was still under anesthetic, mucous membrane biopsies were taken from the middle third of the lower edge of the inferior turbinate. The biopsies were taken with a small, angular right- or left-handed Dabney nasal punch.

The tissue, following removal, was handled in a manner similar to that already described for polyps. It was not possible to study the effect of anesthesia as such on the histamine content of normal nasal mucous membrane. Nor could any reference be found in the literature to suggest that there was

any change in the histamine content of human nasal mucous membrane with various types of anesthesia.

Vascular changes occur in the nasal mucosa during both general and local anesthesia. They may be of a vasodilator type but the majority, and this is especially true of local anesthesia, are of a vasoconstrictor type. If histamine was released from the cells under such circumstances one would expect some degree of vasodilatation to occur, in spite of any antagonistic effects of the premedication and anesthetic agents. In view of this we feel there is no convincing clinical evidence to suggest that there is any significant release of histamine from the cells of the nasal mucous membrane during uncomplicated anesthesia.

C. *Histamine determinations:* The method used for the extraction of histamine from the tissue was essentially that described by Best and McHenry[15] and the assays were performed on atropinized guinea pig ileum. All the histamine values are expressed as gamma of the base per gram of wet tissue.

D. *Histamine values—comparison of wet and dry weights of tissue:* During the recent work of Rose, Entin, and Baxter,[3] but not reported in their paper, a series of simultaneous histamine determinations were carried out on antral mucous membrane and nasal mucous polyps using both wet weight and dry weight of tissue. Wet weight is the weight of freshly removed tissue and dry weight is the weight of tissue that has been reduced to a state of dryness by heating in an incubator.

It was found that for both antral mucous membrane and nasal mucous polyps the histamine determinations on the basis of dry weight as compared with those on the basis of wet weight were consistently from three to five times greater.

This ratio (3-5:1) was found to remain valid in a series of such determinations on mucous polyps removed under varying degrees of nasal symptoms.

The significance of this finding is that a dilution factor does not play a major role in any variation of histamine which may occur in nasal mucous polyps with varying degrees of allergic symptoms. For this reason only wet weight histamine determinations were performed on the material reported here.

E. *Eosinophils:* The following methods were used for the eosinophil determinations:

1. *Peripheral blood:* Blood smears were prepared and stained with Wright's stain. Differential counts were performed and the eosinophils were expressed in terms of percentage. Some of these were checked by direct eosinophil counts, and found to agree.

2. *Nasal smears:* Nasal smears were prepared and stained with Hansel's stain. The interpretation of nasal smears can be somewhat complex and it is difficult to express the number of eosinophils present in terms of percentage. On examination of a smear a general impression is formed rather than an

actual cell count and the results are expressed as a matter of degree (0, +, ++, +++).

It is important in interpreting the nasal smears to scan the whole slide and get a general picture as the eosinophils tend to cluster and may be present only in certain areas. If several fields alone are examined a very misleading interpretation may result.

3. *Nasal tissue:* It was possible to carry out observations on the tissue eosinophils in the allergic cases only as they alone afforded sufficient biopsy material for both histamine determinations and pathologic sections. The sections were prepared by the Surgical Pathology Department of McGill University. They were stained with hematoxylin and eosin.

The interpretation of the tissue eosinophilia is on a similar basis to that of the nasal smears. Here again the general cellular picture of the stroma must be considered and the degree of eosinophilia rather than the percentage is gauged. The whole section must be considered as the eosinophils again tend to cluster and may be very scanty or even absent in some areas.

TABLE I. BLOOD EOSINOPHILS, NASAL SMEAR EOSINOPHILS, AND THE HISTAMINE CONTENT OF NASAL MUCOUS MEMBRANE IN A SERIES OF 30 NORMAL MALE SUBJECTS, AGED 2 TO 12 YEARS, WITHOUT PERSONAL OR FAMILY HISTORY OF ALLERGY

PATIENT	AGE IN YEARS	OPERATIVE PROCEDURE	BLOOD EOSINOPHILIA IN PER CENT	NASAL SMEAR EOSINOPHILIA IN DEGREE	HISTAMINE CONTENT NASAL MUCOUS MEMBRANE IN γ/GRAM
N. G.	4	T&A	1	0	20.5
D. B.	7	T&A	0.5	0	10.4
P. L.	3	T&A	1	0	27.8
K. D.	5	T&A	2	0	13.9
G. M.	6	T&A	1	0	15.0
B. C.	12	T&A	1	0	7.5
G. B.	7	T&A	0	0	54.0
S. F.	4	T&A	1.5	0	28.6
C. N.	5	T&A	0.5	0	15.0
U. C.	4	T&A	0.5	0	20.8
N. C.	3	T&A	0	0	12.1
L. B.	9	T&A	–	–	20.8
W. B.	3	T&A	1	0	22.8
C. W.	5	T&A	3	0	26.3
M. K.	6	T&A	1	0	17.3
D. P.	5	T&A	0.5	0	18.3
R. F.	4	T&A	1.5	0	31.3
R. C.	6	T&A	1	0	13.5
P. L.	7	T&A	0	0	30.0
J. S.	5	T&A	2	0	32.0
G. W.	8	T&A	0	0	32.3
D. M.	4	T&A	1.5	0	15.0
P. F.	5	T&A	–	–	21.8
J. D.	7	T&A	0	0	17.6
A. L.	4	A	2	0	15.8
J. B.	2	T&A	1	0	14.3
M. F.	4	T&A	1	0	21.4
J. P.	8	T&A	0.5	0	22.7
M. T.	3	T&A	1	0	5.0
A. A.	9	T&A	1	0	13.8

Variation: 5.0 to 54.0 γ/gram.
Average: 20.6 γ/gram.

RESULTS

1. *Nonallergic Group.—*

A. *Normal nasal mucous membrane:*

a. Group 1: Birth to 14 years of age.

Males: Thirty individuals were observed ranging from 2 to 12 years of age. The results are shown in Table I. The histamine values varied from 5.0 to 54.0 γ/gram of nasal mucous membrane. The average histamine value was 20.6 γ/gram. The blood eosinophils were within normal values and the nasal smears were negative for eosinophils.

Females: Thirty individuals were observed ranging from 3 to 12 years of age. It will be seen from Table II that the histamine determinations varied from 6.0 to 48.4 γ/gram of nasal mucous membrane. The average histamine value was 18.8 γ/gram. The blood eosinophils were within normal values. The nasal smears were negative for eosinophils.

TABLE II. BLOOD EOSINOPHILS, NASAL SMEAR EOSINOPHILS, AND THE HISTAMINE CONTENT OF NASAL MUCOUS MEMBRANE IN A SERIES OF 30 NORMAL FEMALE SUBJECTS, AGED 3 TO 12 YEARS, WITHOUT PERSONAL OR FAMILY HISTORY OF ALLERGY

PATIENT	AGE IN YEARS	OPERATIVE PROCEDURE	BLOOD EOSINOPHILIA IN PER CENT	NASAL SMEAR EOSINOPHILIA IN DEGREE	HISTAMINE CONTENT NASAL MUCOUS MEMBRANE IN γ/GRAM
J. P.	9	T&A	1	0	20.0
D. B.	6	T&A	0.5	0	19.7
D. R.	4	T&A	2	0	13.3
J. L.	9	T&A	1	0	11.1
P. L.	3	T&A	1	0	15.0
C. D.	4	T&A	2	0	17.3
H. F.	10	T&A	0	0	16.7
J. S.	5	T&A	2	0	9.4
L. C.	6	T&A	0.5	0	11.0
S. P.	6	T&A	1	0	20.0
S. P.	8	T&A	1	0	11.8
D. S.	5	T&A	2	0	16.0
C. S.	6	T&A	1	0	12.0
J. A.	3	T&A	2	0	17.4
C. C.	5	T&A	1	0	48.4
L. A.	6	T&A	1.5	0	12.0
S. D.	4	T&A	0.5	0	31.2
I. D.	6	T&A	2	0	41.7
L. C.	12	T&A	1	0	20.2
S. B.	4	T&A	2	0	14.2
B. M.	11	T&A	1	0	12.5
C. M.	7	T&A	–	–	30.0
A. S.	4	A	0.5	0	17.5
C. P.	3	T&A	1	0	15.0
C. H.	8	T&A	2	0	27.7
H. M.	7	T&A	1	0	6.0
J. M.	9	T&A	2	0	16.2
L. L.	3	T&A	–	–	26.5
L. P.	3	T&A	1.5	0	27.5
M. R.	11	T&A	3	0	6.3

Variation: 6.0 to 48.4 γ/gram.
Average: 18.8 γ/gram.

TABLE III. BLOOD AND NASAL SMEAR EOSINOPHILIA, AND THE HISTAMINE CONTENT OF NASAL MUCOUS MEMBRANE IN A GROUP OF 15 NORMAL MALE SUBJECTS, AGED 18 TO 43 YEARS, WITHOUT PERSONAL OR FAMILY HISTORY OF ALLERGY

PATIENT	AGE IN YEARS	OPERATIVE PROCEDURE	BLOOD EOSINOPHILIA IN PER CENT	NASAL SMEAR EOSINOPHILIA IN DEGREE	HISTAMINE CONTENT NASAL MUCOUS MEMBRANE IN γ/GRAM
M. C.	18	SMR	2	0	21.7
F. B.	33	SMR	1	0	8.3
V. Z.	26	T&A	1.5	0	8.6
C. B.	32	A	2	0	8.6
F. H.	18	T&A	1	0	10.0
J. P.	43	SMR	1.5	0	20.5
W. K.	23	T&A	2	0	11.2
P. C.	35	T&A	2	0	10.0
A. V.	28	T&A	1	0	29.5
L. B.	35	SMR	1.5	0	20.1
F. L.	23	SMR	2	0	5.0
J. D.	38	T&A	2	0	14.2
R. L.	32	SMR	3	0	6.25
T. G.	34	SMR	1	0	4.2
F. B.	37	T&A	2	0	11.6

Variation: 4.2 to 29.8 γ/gram.
Average: 12.7 γ/gram.

b. Group 2. From 15 to 44 years of age.

Males: Fifteen individuals were observed ranging from 18 to 43 years of age. The data are shown in Table III. The histamine determinations varied from 4.2 to 29.8 γ/gram of nasal mucous membrane with an average histamine value of 12.7 γ/gram. The blood eosinophils were within normal values. The nasal smears were negative for eosinophils.

TABLE IV. BLOOD AND NASAL EOSINOPHILIA, AND THE HISTAMINE CONTENT OF NASAL MUCOUS MEMBRANE IN A GROUP OF 15 NORMAL FEMALE SUBJECTS, AGED 15 TO 39 YEARS, WITHOUT PERSONAL OR FAMILY HISTORY OF ALLERGY

PATIENT	AGE IN YEARS	OPERATIVE PROCEDURE	BLOOD EOSINOPHILIA IN PER CENT	NASAL SMEAR EOSINOPHILIA IN DEGREE	HISTAMINE CONTENT NASAL MUCOUS MEMBRANE IN γ/GRAM
W. T.	36	SMR	3	0	19.6
G. N.	15	T&A	0	0	8.2
S. C.	35	T&A	1	0	26.5
E. V.	16	SMR	4	0	7.4
A. D.	21	T&A	2	0	6.25
J. W.	16	SMR	2	0	33.4
M. U.	24	T&A	1.5	0	27.8
D. D.	31	T&A	1	0	14.0
J. N.	18	T&A	2	0	16.5
J. D.	24	T&A	1.5	0	17.0
E. S.	15	T&A	1	0	28.4
G. V.	29	T&A	2	0	38.5
K. O.	39	T&A	2	0	9.6
J. B.	25	T&A	3	0	17.1
J. P.	16	T&A	2	0	8.3

Variation: 6.25 to 38.5 γ/gram.
Average: 18.6 γ/gram.

Females: Fifteen individuals were observed ranging from 15 to 39 years of age. It will be observed (Table IV) that the histamine determinations varied from 6.25 to 38.5 γ/gram of nasal mucous membrane with an average histamine value of 18.6 γ/gram. The blood eosinophils were within normal values. The nasal smears were negative for eosinophils.

c. Group 3. From 45 years of age and older.

This proved to be the most difficult group to obtain and only four cases were observed. These individuals were all in their fifties. Because of the small number of cases, no attempt was made to subdivide them according to sex. The results are shown in Table V.

TABLE V. BLOOD AND TISSUE EOSINOPHILS, AND THE HISTAMINE CONTENT OF NASAL MUCOUS MEMBRANE IN 4 NORMAL SUBJECTS, AGED 50 TO 59 YEARS, WITHOUT PERSONAL OR FAMILY HISTORY OF ALLERGY

PATIENT	SEX	AGE IN YEARS	OPERATIVE PROCEDURE	BLOOD EOSINOPHILIA IN PER CENT	NASAL SMEAR EOSINOPHILIA IN DEGREE	HISTAMINE CONTENT NASAL MUCOUS MEMBRANE IN γ/GRAM
J. M.	M.	59	Turbinectomy	2	0	1.2
E. T.	F.	50	SMR	1.5	0	27.7
E. S.	M.	57	T&A	2	0	55.8
M. K.	F.	50	Antrum*	1	0	27.1
Variation: 1.2 to 55.8 γ/gram.						
Average: 28.0 γ/gram.						

*Radical antrum (Caldwell-Luc).

The histamine determinations in this group varied from 1.2 to 55.0 γ/gram of nasal mucous membrane. The average histamine value was 28.0 γ/gram. The blood eosinophils were within normal values. The nasal smears were negative for eosinophils.

B. *Negative personal, positive family history of allergy:* In order to assess the possible role of a positive family history in an otherwise nonallergic individual, 12 such cases were studied. In the first group, aged 3 to 8 years, the histamine content of the nasal mucous membrane varied from 4.3 to 51.8 γ/gram, with an average value of 19.9 γ/gram (Table VI). The blood eosinophils were within normal, and the nasal smears negative.

TABLE VI. BLOOD AND NASAL SMEAR EOSINOPHILIA, AND THE HISTAMINE CONTENT OF NASAL MUCOUS MEMBRANE IN A GROUP OF 12 NORMAL SUBJECTS, AGED 3 TO 8 YEARS, WITHOUT A PERSONAL, BUT WITH A POSITIVE FAMILY HISTORY OF ALLERGY

PATIENT	SEX	AGE IN YEARS	OPERATIVE PROCEDURE	BLOOD EOSINOPHILIA IN PER CENT	NASAL SMEAR EOSINOPHILIA IN DEGREE	HISTAMINE CONTENT NASAL MUCOUS MEMBRANE IN γ/GRAM
R. C.	M.	7	T&A	2	0	51.8
M. N.	F.	4	T&A	1.5	0	29.3
G. T.	F.	8	T&A	2	0	13.0
W. S.	F.	4	T&A	1	0	14.2
D. M.	M.	3	T&A	2	0	4.3
G. U.	M.	3	T&A	1.5	0	10.4
W. T.	M.	5	T&A	0	0	12.6
K. O.	F.	4	T&A	1	0	20.8
B. O.	M.	4	T&A	2	0	23.3
Variation: 4.3 to 51.8 γ/gram.						
Average: 19.9 γ/gram.						

TABLE VII. BLOOD AND NASAL SMEAR EOSINOPHILIA, AND THE HISTAMINE CONTENT OF NASAL MUCOUS MEMBRANE IN A GROUP OF 3 NORMAL SUBJECTS, AGED 19 TO 49 YEARS, WITH A NEGATIVE PERSONAL AND A POSITIVE FAMILY HISTORY OF ALLERGY

PATIENT	SEX	AGE IN YEARS	OPERATIVE PROCEDURE	BLOOD EOSINOPHILIA IN PER CENT	NASAL SMEAR EOSINOPHILIA IN DEGREE	HISTAMINE CONTENT NASAL MUCOUS MEMBRANE IN γ/GRAM
N. B.	F.	22	T&A	2	0	14.3
A. P	M.	49	A	1	0	5.8
L. L.	F.	19	T&A	1.5	0	5.8

A smaller group of three patients, aged from 19 to 49, showed a range of 5.8 to 14.3 γ/gram, as shown in Table VII. The average was 8.6 γ/gram. Blood eosinophils were normal, and the nasal smears negative for eosinophils.

C. *Chronic infection—negative personal and family history of allergy:* As shown in Table VIII, three cases with ages varying from 24 to 33 years of age were available. The tissues were removed during radical antral surgery for chronic infection which had no demonstrable allergic component. The histamine values were all within the limits previously obtained for this age group, the average being 20.9 γ/gram. Again the blood eosinophils were within normal, and the nasal smears negative for eosinophils.

TABLE VIII. BLOOD AND NASAL SMEAR EOSINOPHILIA, AND THE HISTAMINE CONTENT OF NASAL MUCOUS MEMBRANE IN A GROUP OF 3 NORMAL SUBJECTS, AGED 24 TO 33 YEARS WITH A NEGATIVE PERSONAL AND A NEGATIVE FAMILY HISTORY OF ALLERGY

PATIENT	SEX	AGE IN YEARS	OPERATIVE PROCEDURE	BLOOD EOSINOPHILIA IN PER CENT	NASAL SMEAR EOSINOPHILIA IN DEGREE	HISTAMINE CONTENT NASAL MUCOUS MEMBRANE IN γ/GRAM
E. W.	F.	27	Antrum	2	0	37.5
B. J.	M.	33	Antrum	1	0	24.1
O. G.	M.	24	Antrum	2	0	1.32

D. *Chronic infection—positive nasal allergy:* Two allergic cases with nasal mucous polyps and an associated antral infection were observed. These patients had radical sinus surgery performed and the histamine values of the antral mucosa were 40.4 and 9.3 γ/gram, respectively. These values are similar to Group 2 normal (4.2 to 38.5 γ/gram).

2. *Allergic Group.—*

E. *Allergic human nasal mucous membrane—nasal mucous polyps:* In all, 56 cases with nasal polyps were studied (35 males, 21 females) and 59 separate operative procedures were performed. The cases ranged from 15 to 70 years of age. Except for one case, they all had definite proved allergy. The one exception, although no definite allergy could be proved, was strongly suggestive of such an etiology. Half of the cases had a positive family history while the other half had a negative family history of allergy. They were all cases of perennial allergy with symptoms ranging from 6 months to 45 years.

The histamine content of the polyps varied from 2.6 to 48.9 γ/gram with an average of 14.3 γ/gram (Table IX). This range is broader than Group 2 normal (4.2 to 38.5 γ/gram) but the average values are quite similar (Group 2 normal, 15.7 γ/gram).

From these figures it appears that there is no significant difference by wet weight of tissue in the histamine content of nasal mucous polyps when compared as such with that of normal mucous membrane. This confirms the work of Buhrmester and Wenner.[2] However, in such comparisons no provision was made for the degree of the allergic reaction present at the time the tissues were removed. In this study the degree of nasal signs and symptoms (degree of the allergic reaction) present at the time the nasal polyps were removed was recorded and efforts were made to remove tissue during various phases of the disease process.

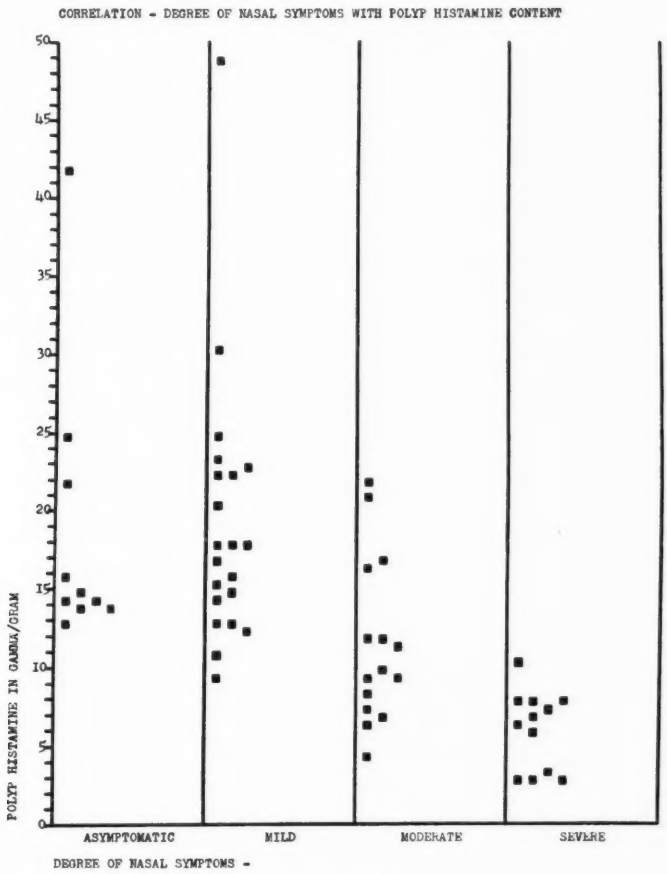

Fig. 1.

An attempt to correlate the degree of nasal signs and symptoms with the histamine content of the polyps is shown in Fig. 1. It is obvious from this figure that with increasing degrees of nasal signs and symptoms there is a decrease in the histamine content of the nasal polyps; that is, with an increase in the severity of the allergic reactions, the histamine content of the polyp is decreased.

The opportunity arose in two patients to obtain biopsy material during different phases of their nasal signs and symptoms. The correlation between

TABLE IX. DATA ON 56 SUBJECTS WITH BASAL MUCOUS POLYPS, AND A POSITIVE HISTORY OF ALLERGY. THE ALLERGIC SYMPTOMS AND SIGNS, BLOOD, NASAL SMEAR AND TISSUE EOSINOPHILIA, AND HISTAMINE CONTENT OF POLYPS ARE SHOWN

PATIENT	SEX	AGE IN YEARS	PERSONAL HISTORY OF ALLERGY	FAMILY HISTORY OF ALLERGY	DURATION OF NASAL SYMPTOMS IN YEARS	DEGREE OF NASAL SYMPTOMS AND SIGNS	BLOOD EOSINOPHILS PER CENT	NASAL SMEAR EOSINOPHILS	TISSUE EOSINOPHILS	HISTAMINE CONTENT OF POLYPS γ/GRAM
S. H.	F.	68	Yes	No	10	Mild	1	++	+++	30.4
M. G.	F.	35	Yes	No	7	Mild	1	0	+	12.6
M. G.	F.	35	Yes	No	7	Mod.	4	++	++	9.1
E. W.	M.	37	Yes	Yes	14	Mild	1	0	0	23.1
F. S.	F.	67	Yes	Yes	25	Mild	3	+	+++	22.0
F. S.	F.	67	Yes	Yes	25	Severe	6	+	+++	6.2
F. S.	F.	67	Yes	Yes	25	Severe	6	++	+++	5.8
F. B.	F.	70	Yes	No	1	Nil	1	0	−	14.0
A. B.	F.	60	Yes	No	45	Mild	0	+	++	12.5
A. B.	F.	60	Yes	No	45	Mild	9	+	+	17.5
K. G.	F.	55	Yes	No	12	Mod.	7	+	−	16.1
A. S.	M.	28	Yes	No	8	Severe	3	0	+	6.67
B. B.	F.	15	Yes	Yes	1	Nil	2	+	+	41.5
A. A.	M.	48	Yes	No	40	Nil	3	++	+++	13.7
L. F.	M.	46	Yes	−	10	Nil	1	0	−	14.2
J. M.	M.	29	Yes	−	6	Nil	2	+	++	14.7
M. H.	M.	54	Yes	No	30	Nil	1	0	0	10.5
J. H.	M.	63	Yes	No	31	Nil	2	+	−	13.8
A. G.	M.	53	Yes	−	25	Mod.	1	+	+	9.6
B. F.	M.	39	Yes	No	6	Mild	5	++	+	17.8
H. W.	M.	42	Yes	No	30	Severe	8	+	+	10.2
W. R.	M.	50	Yes	No	22	Nil	0	−	0	24.8
A. S.	M.	69	Yes	No	6	Mod.	1	−	+	7.3
U. B.	F.	57	Yes	Yes	10	Mod.	4	0	+	6.8
G. W.	M.	45	Yes	Yes	15	Mild	3	++	+	15.3
W. S.	F.	55	Yes	Yes	1	Severe	2	0	++	3.8
Y. V.	F.	38	Yes	No	8	Mild	6	+	+	20.0
S. R.	F.	46	Yes	Yes	2	Severe	2	+++	+++	7.8
S. R.	F.	46	Yes	Yes	2	Mild	3	+++	−	22.2
A. W.	F.	61	Yes	Yes	9	Mild	−	−	+++	14.0
A. T.	M.	41	Not proved	No	8	Mild	1	+	+	14.7
J. C.	M.	27	Not proved	No	11	Mild	1	0	+	16.7
B. S.	F.	39	Not proved	Yes	7	Severe	0.5	+	++	7.7
C. V.	M.	19	Not proved	Yes	4	Mild	1	+	+	22.7
A. S.	M.	48	Yes	Yes	12	Nil	3	+	+	15.8
M. S.	M.	48	Yes	No	1	Mod.	2	+++	+	11.7
A. L.	M.	41	Yes	Yes	6	Nil	2	+	+++	21.9
S. T.	M.	17	Yes	Yes	2	Mod.	20	+	++	16.7

D. D.	M.	68	Yes	Yes	40	Mod.	–	–	++	11.6
M. C.	F.	52	Yes	Yes	14	Severe	2	++	+++	7.4
M. P.	M.	24	Yes	Yes	3 mo.	Mild	3	+	+++	48.9
L. P.	M.	48	Yes	No	1	Mild	1	0	++	15.9
J. L.	M.	34	Yes	Yes	10	Mod.	1	+	+	11.4
R. S.	M.	54	Yes	Yes	10	Severe	2	++	+++	2.63
R. C.	M.	42	Yes	Yes	6 mo.	Mild	11	+	+	20.0
A. W.	M.	72	Yes	Yes	30	Mod.	6	+	+	9.26
P. Z.	M.	28	Yes	No	3	Mod.	1	0	+	20.9
E. Y.	F.	43	Yes	Yes	12	Nil	2	0	0	12.7
D. J.	F.	18	Yes	Yes	14	Mod.	2	+	–	21.8
R. T.	M.	50	Yes	–	6 mo.	Mod.	2	0	0	6.0
L. H.	M.	30	Yes	No	1	Mod.	1	++	+	4.2
S. S.	M.	67	Yes	Yes	15	Mild	1	0	+	9.0
R. W.	M.	53	Yes	No	1	Mild	2	+	+++	24.8
J. W.	M.	49	Yes	No	27	Mod.	2	+	+	8.38
B. G.	F.	57	Yes	No	17	Severe	3	+	+	7.8
J. P.	M.	50	Yes	No	17	Severe	4	+	++	3.1
P. P.	F.	36	Yes	No	1	Mild	1	0	+	17.5
P. B.	M.	50	Yes	–	15	Severe	3	+	+	2.6
T. K.*	F.	40	Yes	–	3	Mod.	–	–	–	108.0

Variation: 2.6 to 48.9 γ/gram.
Average: 14.3 γ/gram.

*Not included in general series, but discussed in text.

the polyp histamine content and the signs and symptoms are shown in Figs. 2 and 3. It will be noted that the histamine content of the tissue was considerably lower when symptoms were present.

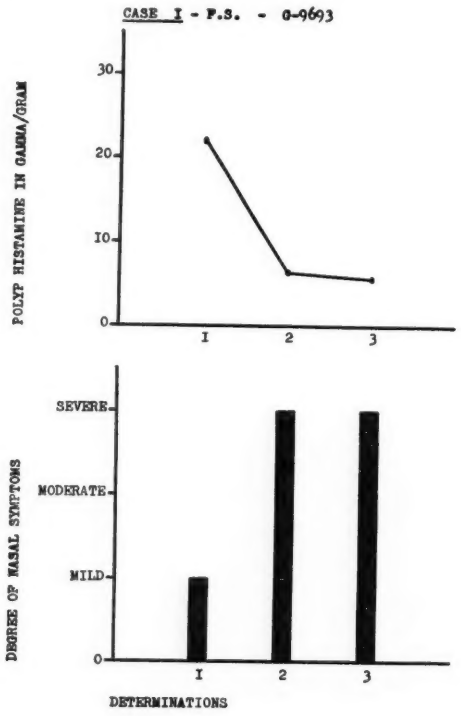

Fig. 2.

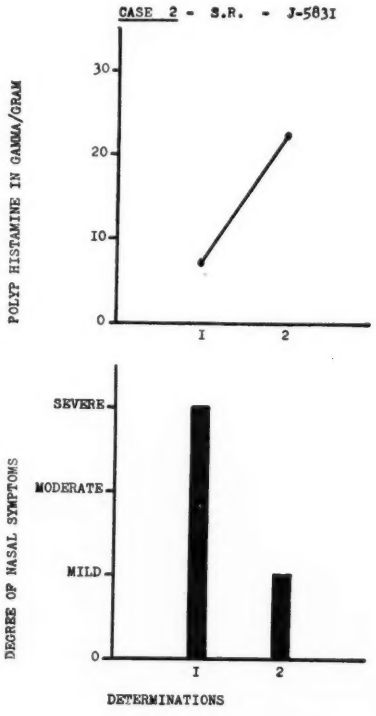

Fig. 3.

F. *Observations on blood and tissue eosinophils:* As already noted, the blood eosinophils were within normal limits and no eosinophils could be found in the nasal smears of the group of normal cases. Unfortunately, insufficient biopsy material was obtained for both histamine and tissue eosinophilia, as a result of which the latter was not done.

In the allergic cases, however, alterations were observed. There was a blood eosinophilia in many, and varying degrees of both tissue and nasal smear eosinophilia were the rule in the majority of cases. From the data obtained, which are presented in a series of figures, it will be seen that there was no correlation between the degree of nasal symptoms and the percentage of blood eosinophilia (Fig. 4), the degree of nasal smear eosinophilia (Fig. 5), or the degree of tissue eosinophilia (Fig. 6). Furthermore, no correlation could be found between simultaneous observations on the eosinophil content of the blood, nasal smears, or tissues, nor was there a correlation between the histamine content of the polyps and the degree of tissue eosinophilia.

DISCUSSION

It is clear from the foregoing results that there is considerable variation in the histamine content of normal nasal membrane. The average values given at the bottom of the various tables showed a deviation of from 1.2 to 55.8

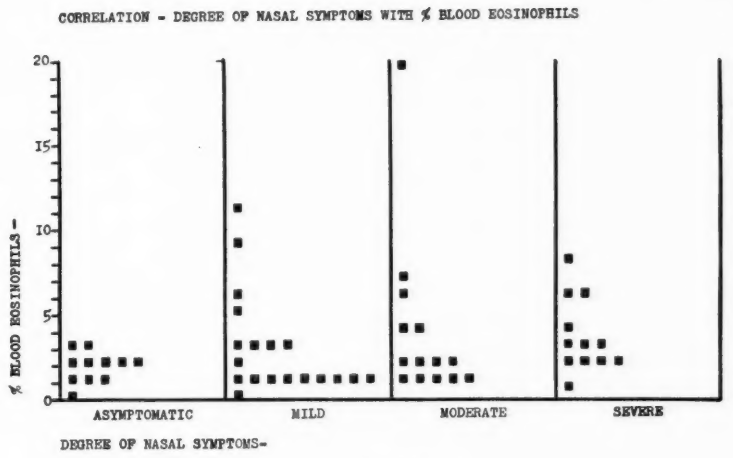

Fig. 4.

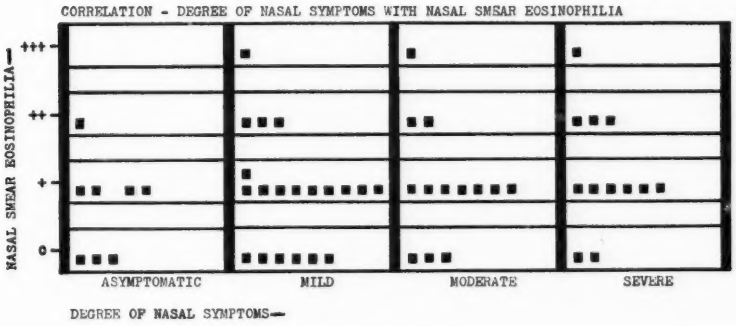

Fig. 5.

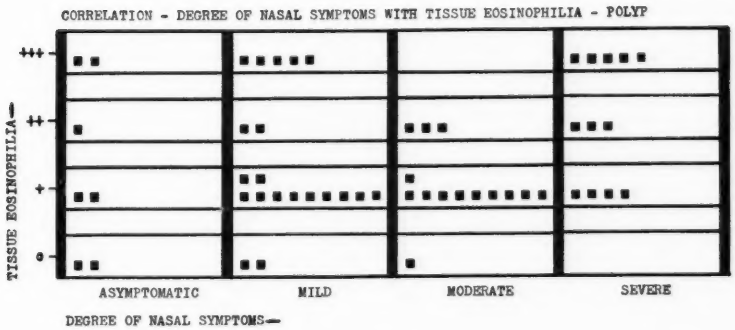

Fig. 6.

γ/gram. The average value for each group was 19.7, 15.7, and 28.0 γ/gram, respectively, for Groups 1, 2, and 3. When the determinations were arranged according to the sex of the various subjects there was no significant correlation. These findings are consistent with observations on other tissues such as lung, spleen, and skin, as found by Rose and associates.[3]

Two extremely high values, 83.3 and 198.4 γ/gram, were found in two male patients in Group 1. The personal and family histories were negative for allergy and the patients were asymptomatic at the time the material was taken. The significance of these latter findings is not clear, and these figures are not included in Table I. As was to be expected, the blood eosinophils were within normal limits, and the nasal smears were negative for eosinophils in this group of normal subjects. Furthermore, there was little difference in the histamine content of nasal mucous membrane in nonallergic patients with such complications as a positive family history of allergy or accompanying infection, although the numbers of available cases in these two latter groups were small.

Since the histamine content of nasal polyps in allergic individuals appeared to vary within the range found for that of normal mucous membrane, this could fit in with the theory that sensitization as such does not increase the histamine content of tissues. The latter finding has been confirmed many times in animal experimentation, and was clearly stated by Dale.[25] Thus the combination of antigen with antibody does not form histamine, but releases preformed histamine from the cells into the extracellular spaces. The present work appears to be in agreement with this concept as well since the more severe the allergic symptoms, the lower the histamine content of polyps as shown in Fig. 1. Similar findings have been reported by Pellerat[26] for the skin following wheal production.

During this study, one allergic case with moderate nasal signs and symptoms was observed. A polyp was removed and found to have a value of 108 γ/gram. As already noted two normal male children were observed with nasal mucous membrane histamine values of 83.3 and 198.4 γ/gram. Rose, Entin, and Baxter[3] in their small series of nasal tissues, obtained two such values for antral mucous membrane (101 and 140 γ/gram). It was thought initially that such high values might be associated with allergic tendencies, chronic infection, or both. This has been shown not to be the case in the present studies. The exact significance of these findings is not clear at this time.

There is one possible mechanism which has not been adequately explored relative to histamine in relation to allergy. The blood histamine is quite constant in normal individuals but varies considerably, often well above normal in patients with various forms of allergic phenomena.[27] It may, therefore, be that there is a greater turnover or production of histamine in patients with allergy as compared to nonallergic subjects.

Since there is little evidence to support the view that the eosinophil carries histamine,[24 28] it is not surprising that no correlation could be demonstrated between the histamine content of polyps and the degree of eosinophilia. Neither a blood nor nasal smear eosinophilia could be demonstrated in normal subjects although the histamine content of the tissue varied within

limits similar to those found in the tissues from allergic patients. In the latter group a blood eosinophilia was the rule for the majority. Unfortunately, the amount of tissue obtained in the group of normal subjects did not allow for both histamine determinations and sections for staining.

From these observations, however, it appears that the demonstration of eosinophils in nasal smears is good presumptive evidence of nasal allergy. Since no observations were made in this study on normal nasal tissues, no conclusions could be drawn on the significance of the tissue eosinophilia observed in the majority of allergic patients. However, it has been known for years that allergic reactions are associated with a local tissue eosinophilia.[12, 17, 18, 19, 20, 21] In a study of almost one hundred cases, Cowie and Jimenez[22] found that simultaneous differential counts on blood smears and nasal smears did not show any definite relationship to each other. This is confirmed in the data presented in the present study. It is possible, however, that, as with blood, there may be a considerable diurnal variation in tissue eosinophilia, and this might alter the interpretation of these findings.

SUMMARY

1. There is a wide variation in the histamine content of normal nasal mucous membrane. There are slight differences in this variation according to age and sex groups, but the over-all normal variation is from approximately 5 to 50 γ/gram of histamine by wet weight of tissue.

2. In normal children the males have a slightly higher average nasal mucous membrane histamine value than the females, while in the adults the females have a significantly higher average histamine value than the males. The average female histamine value for children and adults is approximately the same.

3. The nasal mucous membrane of individuals with inherited allergic tendencies, chronic infections, and chronic infections associated with nasal allergy have histamine values within the normal range of variation.

4. The variation of the histamine content of nasal mucous polyps (allergic nasal mucous membrane) on the basis of wet weight is similar to that of the normal nasal mucous membrane and the average histamine values are almost identical.

5. The histamine content of nasal mucous polyps is consistently lower during the presence of active nasal allergy as compared to quiescent states.

6. In both normal nasal mucous membrane and nasal mucous polyps an occasional high histamine value is encountered, the exact significance of which is not clear.

7. The finding of eosinophils in nasal smears and nasal tissue is good presumptive evidence for the existence of nasal allergy but it is not a definite diagnostic criterion.

8. There is no correlation between simultaneous observations on blood, nasal smear, and tissue eosinophilia in cases of nasal allergy.

9. There is no correlation between the degree of nasal allergy and the blood, nasal smear, and tissue eosinophilia.

10. There is no correlation between the degree of tissue eosinophilia and the histamine content of the nasal mucous polyps.

The authors are indebted to Dr. W. J. McNally, Otolaryngologist-in-Chief, Royal Victoria Hospital, Montreal, for provision of patients from the Department of Otolaryngology, and for his interest and guidance. Thanks are due to Mrs. E. V. Harkness for the histamine assays.

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THE MUCOPROTEINS OF THE NASAL MUCOSA OF ALLERGIC PATIENTS BEFORE AND AFTER TREATMENT WITH CORTICOTROPIN

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WHILE corticotropin and cortisone produce profound clinical and metabolic effects in a variety of conditions, histologic studies have shown surprisingly little change attributable to these agents. In 1950 Hench[1] stated that adrenal corticosteroids affect the pathologic physiology rather than the pathologic anatomy or the etiologic factors. Bordley,[2] who studied polypoid tissue removed from five allergic patients treated with corticotropin, was unable to demonstrate significant histologic changes. A recent study[3] of the nasal mucosa of hay fever patients, before and after corticotropin therapy, confirmed that there was no characteristic difference between pretreatment and posttreatment specimens if routine histologic methods were used.

In the present study, a somewhat closer analysis of certain features of normal and allergic nasal mucosa has been attempted. For this purpose, histochemical methods for demonstrating carbohydrate-protein conjugates were employed. These compounds are characteristic components of the connective tissue which can be detected by methods devised by Hotchkiss[4] and others.[5, 6] It was decided to examine the tissues obtained in our 1950 studies as well as additional specimens taken under similar conditions during the 1951 ragweed season with these techniques.

METHODS

A. *1950 Biopsies.*—The details of the experimental procedure of the 1950 study have been previously described.[3] In brief, biopsies of the nasal mucosa of ragweed-sensitive patients were made on August 24, before treatment with corticotropin was started, and on September 1, at the end of four days of treatment with 80 mg. corticotropin per day. The tissues removed from these patients, as well as from eight untreated hay fever patients, were fixed by freezing and drying.

B. *1951 Biopsies.*—

1. *Selection of patients:* For "normal controls" tissues were obtained from the turbinates of six volunteers who had no history of allergic symptoms, gave no positive skin reactions to grass or ragweed pollen, and at the time of the biopsies had no evidence of nasal inflammation. The individuals selected were all men between the ages of 18 and 35.

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This study was supported in part by grants from the Asthmatic Children's Aid of Chicago and the American Cancer Society.

Presented in part before the Eighth Annual Meeting of The American Academy of Allergy, Chicago, Ill., February, 1952.

Received for publication May 15, 1952.

To supplement the material available from the previous study, fifteen patients from the Allergy Clinic of the University of Illinois College of Medicine were chosen because ragweed pollen sensitivity was the major cause of their symptoms. Four of the fifteen had perennial respiratory allergy in addition to seasonal ragweed pollinosis. There were six men and nine women in the group. Twelve of the patients ranged in age from 18 to 39, two were 45, and one was 75.

Five of the fifteen ragweed-sensitive patients were selected at random for placebo medication. Seven of the remaining ten were hospitalized for treatment with corticotropin, while three received the hormone as outpatients.

2. *Medication:* Three hundred fifteen units of corticotropin were injected intramuscularly during the four-day period from August 27 to August 31, using the following general schedule: 25 units every six hours during the first two days, 20 units every six hours during the third day, and 15 units every six hours during the fourth day. The biopsies were made one to three hours after the final dose. During the ensuing twenty-four hours 20 units of corticotropin were given intramuscularly in divided doses.

The ten treated patients were placed on a salt-poor diet and received 10 gr. of potassium chloride in enteric-coated tablets three times daily. This medication was likewise given to the five untreated patients.

3. *Preparation of tissue for histologic study:* Tissues from the inferior turbinates of the six nonallergic individuals were obtained at various periods from April to August, 1951. The first biopsies from the fifteen allergic individuals were made on August 24, three days before starting corticotropin injections. The second biopsies in these treated patients were made on August 31. The five untreated allergic patients had the second biopsies on the morning of September 1. After five minutes of topical anesthesia with 2 per cent Pontocaine, a section about 2 mm. square was removed with a Myles anterior-biting forceps from the anterior overhang of the middle turbinate. Personal experience of one of the authors indicates that with good technique and a sharp instrument the discomfort of the procedure is small.

Within seconds after removal, the tissues were fixed by rapid freezing in iso-pentane chilled by liquid nitrogen to a temperature of –150° C. to –160° C. according to the Altmann-Gersh technique.[7] Subsequently, the tissues were dehydrated in vacuo at about –30° C. By this method protein denaturation is minimized and shifts in inorganic and organic tissue components are lessened. Enzyme activity is arrested.

After complete dehydration, the tissues were prepared for sectioning by infiltration with paraffin (m.p. 52° –56° C.). Sections were cut at thicknesses ranging from 12 to 2 microns. They were stained for routine histologic study with hematoxylin and eosin and Giemsa and, for the study of mucoproteins, with the Hotchkiss technique.[4] The tissues from each patient taken before and after treatment were stained simultaneously when the Hotchkiss method was used.

The technique for staining carbohydrate-protein conjugates (mucoproteins or glycoproteins) was independently described by McManus,[5] Lillie,[6] and Hotchkiss.[4] The last-named author gave the stain chemical significance by explaining its mode of action. By the action of periodic acid, adjacent hydroxyl (or hydroxylamino) groups in sugar residues are oxidized to aldehyde. These give pink, red, or purple compounds with leukofuchsin. With suitable control procedures[8] this technique may be made relatively specific for glycoproteins in tissues.

RESULTS

For purposes of clarity it is useful to describe the various structures stained under the following headings: (1) ground substance, (2) cellular elements of the connective tissue, and (3) mucous glands.

A. *The Nasal Mucosa of Nonallergic Persons.*—

Hematoxylin and eosin and Giemsa stains: 1. The ground substance is the substrate in which the various organized structural elements, i.e., cells, fibers, blood vessels, glands, etc., are embedded. It is coextensive with, but different from, the tissue fluid. The material composing the ground substance does not commonly stain with routine stains, but remains as a clear background against which the stained elements are seen. Below the epithelium is an acellular zone of condensed, fibrillar material of width varying from 5 to 20 microns which together with the ground substance constitutes the "basement membrane."

2. The connective tissue elements of the normal nasal mucosa need only brief mention here. The submucosa, usually referred to as the "tunica propria," contains primarily typical fibroblasts, characterized by an elongated, spindle-shaped nucleus and cytoplasm stained faintly with both stains. Eosinophils are rarely present and plasma cells are few in number.

3. The mucous glands as stained with routine methods are described in histologic texts.

Hotchkiss stain: Materials containing polysaccharides or polysaccharide-protein conjugates stain from a pale pink to a deep purple with the Hotchkiss method.

In their study of ground substance with the Hotchkiss method, Gersh and Catchpole[8] characterize this material as follows:

> Intercellular substances fall into at least two categories. The first of these includes various substances summarized by the term "tissue fluid": water containing proteins, crystalloids, metabolites, and gases. These are related primarily to the plasma, whose composition they largely reflect, and only secondarily to connective or other tissues. The second category includes the microscopically non-fibrillar ground substance(s) whose most characteristic component is a glyco-protein (that is, a protein which contains a carbohydrate moiety as an integral part of its structure).

The ground substance adjacent to certain structures consists of a more deeply staining region which is usually termed the "basement membrane" of the organized structure on which it impinges. In the nasal mucosa the ground

substance is condensed to form basement membranes at the junction of the epithelium with the tunica propria, about the endothelium of capillaries, and at the periphery of the glands of the submucosa.

The collagen fibers and the cytoplasm of cells stain more deeply with the Hotchkiss method than the ground substance in which they are imbedded. The

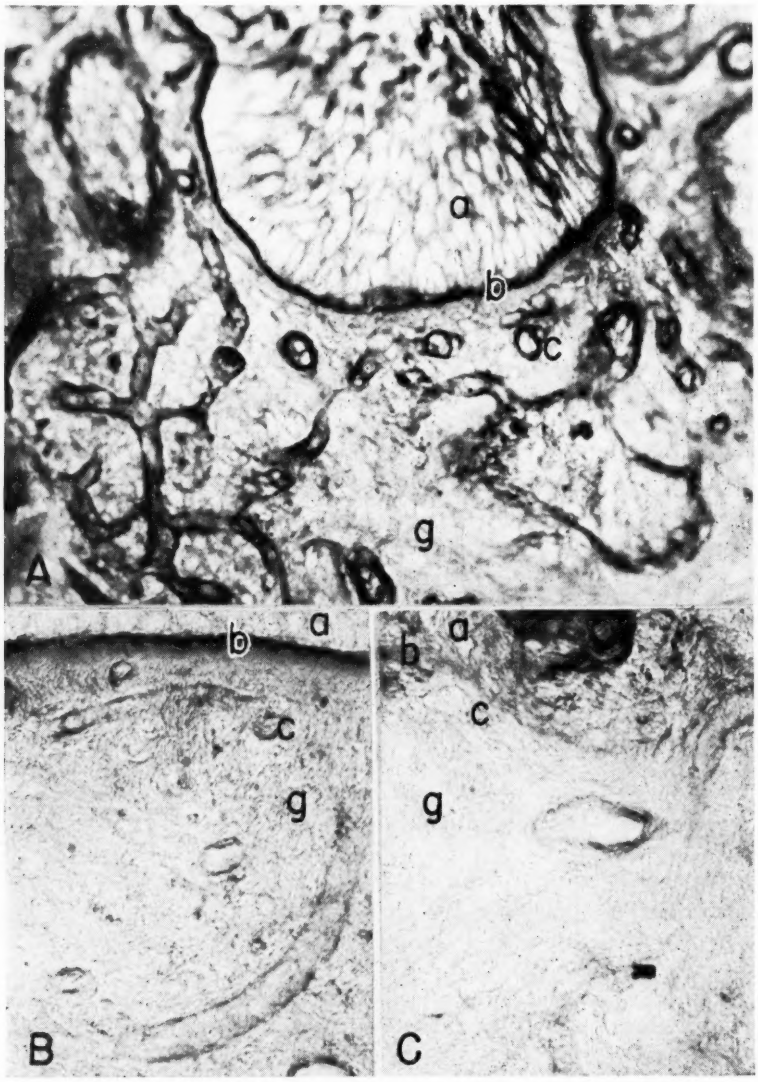

Fig. 1.—*A* shows the nasal mucosa of a nonallergic person. *B* and *C* are sections from the mucosa of one patient with hay fever, *B* on August 24 during moderate symptoms, *C* on September 1 during severe symptoms. The major differences between the normal and the allergic nasal mucosa as well as the progressiveness of the changes as symptoms increase were as follows:

1. The interepithelial cement (*a*) lost staining intensity progressively.

2. The subepithelial (*b*) and the pericapillary (*c*) basement membranes became progressively narrower and less intensely stained to a point where visibility was almost completely lost (*C*). Small capillaries are difficult to resolve in the photograph.

3. The ground substance (*g*) became more edematous and less deeply stained.

Sections were cut at 4 microns and simultaneously stained by the Hotchkiss method (×500).

cell nucleus, whether its origin be epithelium, connective tissue, or blood, does not stain with this method. It appears within the cell as a clear, sharply defined area corresponding to its particular shape.

1. The ground substance: When stained with the Hotchkiss method the ground substance of normal nasal mucosa is pale pink and homogeneous. The basement membrane bordering the surface epithelium appears to be composed of two zones (Fig. 1, *A*). The outer one, adjacent to the base of the innermost row of epithelial cells, consists of a densely staining area several microns in width which, under the oil immersion lens (900×), appears as closely packed, coarse granules imbedded in the ground substance. Adjacent to this deep-staining, dark violet-colored zone is a lighter staining bandlike area 10 to 20 microns in width which gradually merges with the general ground substance. This inner zone is distinguishable from the ground substance by its greater optical density, its deeper staining properties, and its homogeneous, acellular appearance. Under the oil immersion lens (900×) this zone appears to contain fine fibrils which run parallel to the long axis of the epithelial cells. The two zones described are spatially identical with the basement membrane seen in hematoxylin and eosin-stained sections.

At the junction of the capillary endothelium the ground substance appeared similarly differentiated into a densely staining, homogeneous zone surrounding each capillary (Fig. 1, *A*). This is the capillary basement membrane, referred to by some as the pericapillary sheath. Similarly, adjoining each gland, the ground substance appeared as a homogeneous, deeply stained basement membrane surrounding the periphery of the gland.

With the Hotchkiss method the collagen fibers of the normal mucosa stain more deeply than the ground substance in which they are imbedded. They are arranged in loose bundles typical of areolar connective tissue.

2. Connective tissue cellular elements: The cytoplasm of the fibroblasts stains a little more deeply than the ground substance. Occasionally a cell contains a few granules, staining deeply purple, of such small size that they can barely be resolved with highest magnifications. One may also find a few stellate and irregularly shaped connective tissue cells with many large, deeply staining cytoplasmic granules of uniform size.

3. The mucous glands: The mucous-secreting glands are sharply demarcated from the surrounding ground substance by their narrow but deeply staining basement membranes. Between adjoining epithelial cells composing the gland there is a homogeneous deeply staining purplish material, the intercellular cement. A similar interepithelial cement is present between adjoining cells covering the surface of the mucosa. Present in clumps or individually throughout the gland, but concentrated mainly near its lumen, are many large, dense, dark purple-staining mucigen granules.

B. *Untreated Allergic Patients Exposed to the Specific Antigen.*—

Hematoxylin and eosin and Giemsa stains: 1. As studied with these staining methods, the allergic nasal mucosa was distinguished from the nonallergic by the presence of edema in the tunica propria and differences in the cellular

elements. The edema was evidenced primarily by separation of tissue elements, especially of collagen fibers and the presence of artifacts due to the freezing and drying method of fixation. These artifacts consist of small round or oval, nonstaining areas which were due to the formation of ice crystals in the process of rapid freezing. In severely edematous tissues the cytoplasm of cells may have a finely vacuolated appearance due to the similar formation of intracellular ice crystals. The tissues removed on August 24 were less edematous than those obtained from the second biopies later in the season.

2. The connective tissue cells: The cells found in the allergic nasal tissues differ greatly from those seen in nonallergic mucosa. There are first those cells classified by most, but not all, workers as of hematogenous rather than of tissue origin. Of these, the eosinophils, as noted in a previous report,[3] were concentrated primarily in the tunica propria immediately below the epithelial layer. The number per high-power field (×500) in the tissues of the first biopsies (August 24) as compared with those of the second (September 1) showed a random variation in the 1951 group similar to that described earlier.[3] As many as two hundred per high-power field were observed in one of the tissues removed from a patient as contrasted with two or three cells in a similar area of tissue from the same patient at a different period. Their concentration did not vary with the duration of exposure or concentration of pollens; in some individuals the tissue removed on August 24 was richer in eosinophils than that of September 1, while in others the reverse was true.

Tissue eosinophils differed in morphology from those found in circulating blood. Many of the cells were irregularly shaped and frequently were stretched out as if in ameboid movement. The nucleus was often round or oval and frequently contained a nucleolus. Two or more nuclei were quite common in large cells with typical eosinophilic cytoplasmic granules characteristic of the eosinophil. In addition to typical lymphocytes there were round cells with the morphology and staining characteristics of plasma cells. The latter were frequently much larger than those usually seen in circulating blood.

In both the first and second of the paired biopsies, the connective tissue cells differed from those of nonallergic nasal mucosa by the presence of a variety of immature or mesenchymal type cells. In addition to the elongated, branching phagocytes there were many large, star-shaped, oblong or irregularly shaped cells with an abundance of homogeneous cytoplasm which stained much more deeply than that of the fibroblasts. Their nuclei were round or oval and frequently contained one or more nucleoli. Finally, there were many cells of varied shapes, oval, octagonal, and round, with multiple nuclei, numbering from 2 to 6, each containing one or more nucleoli, and whose cytoplasm stained homogeneously pale pink to pink-violet with hematoxylin and eosin. These appeared to be multinucleated mesenchymal cells.

3. The mucous glands: With hematoxylin and eosin and Giemsa stain the mucous glands of allergic nasal tissue could not be differentiated from those of nonallergic tissue.

The Hotchkiss stain:

1. Ground substance: The ground substance of the allergic nasal mucosa obtained after exposure to the specific allergen differed from the nonallergic nasal tissue by a lessening of its affinity for the acid-leukofuchsin dye. While the general decrease in staining intensity of the allergic tissue, as compared with normal nasal mucosa, was definite, the most marked changes were observed in the stainability and width of the basement membranes (Fig. 1, *A*, *B*, and *C*). The first change observed in the subepithelial basement membrane during periods of mild symptoms was in the broad inner layer. The density and depth of staining in this region decreased so that in many cases the area was indistinguishable from the rest of the ground substance. The mucosa obtained during severe symptoms showed greater alteration in the basement membrane; the deeply staining granular zone just below the epithelium became less distinct, until, in the patients with very severe symptoms, this area under high power (×500) was seen as a very fine, granular line whose integrity between the epithelium and the tunica propria was not always maintained (Fig. 1, *C*).

The basement membranes around the capillaries and the glands showed a similar progressive decrease in width and stainability. In patients with severe symptoms a very thin linear purple zone frequently incompletely surrounded the capillary endothelium (Fig. 1, *C*). The changes seemed to correspond with the duration of exposure and possibly with the dose of pollen that the patients had experienced (compare Fig. 1, *B* and *C*). The histologic changes in the tissues of the second biopsies of all the thirteen untreated patients, i.e., the changes when symptoms were more severe, were more marked than those present in corresponding tissues from the first biopsies taken earlier in the season when symptoms were milder.

TABLE I. COMPARISON OF NASAL BIOPSIES TAKEN FROM 13 INDIVIDUAL RAGWEED-SENSITIVE PATIENTS ON AUGUST 24 AND ON SEPTEMBER 1, 1950

		NUMBER OF PATIENTS SHOWING EFFECTS:		
		INCREASED	DECREASED	UNALTERED
Basement membrane	Subepithelial	1	8	4
	Capillary	0	8	5
Ground substance	Edema	12	0	1
	Stainability	1	4	8
Interepithelial cement		0	4	9
Mucous glands—mucigen granules		0	5	8

On September 1, the clinical symptoms were at their height. The patients were not treated. For each patient, sections from the later as compared with the earlier biopsy were stained simultaneously and scored with respect to staining intensity, degree of edema, number of mucigen granules, etc. Note that the predominant changes were in the subepithelial and pericapillary basement membranes (which became thinner) and in the edema of the ground substance.

The collagen fibers imbedded in the ground substance were frequently present as separated strands rather than bundles. The tendency for parallel arrangement of fibers in bundles was lost in tissues that were markedly

edematous with a resulting appearance of a thin lacework of single fibers. The diameters of the individual fibers appeared smaller in allergic compared with those of nonallergic tissues.

2. Cells: The cement substance between adjacent cells of the surface epithelium, as well as that between the parenchymal cells of the mucous glands, stained less deeply with the Hotchkiss method in allergic than in nonallergic nasal mucosa. The difference was best observed in the interepithelial cement of the gland cells and was proportional to the reduction in the staining properties of the ground substance. In those cases where the changes were great, the interepithelial cement appeared as a thin, unstained, linear area between adjoining cells.

TABLE II. COMPARISON OF NASAL BIOPSIES TAKEN FROM 22 INDIVIDUAL RAGWEED-SENSITIVE PATIENTS TREATED WITH CORTICOTROPIN FOR FOUR DAYS DURING THE HEIGHT OF THE RAGWEED SEASON OF AUGUST, 1950

		NUMBER OF PATIENTS SHOWING EFFECTS:		
		INCREASED	DECREASED	UNALTERED
Basement membrane	Subepithelial	20	1	1
	Capillary	20	1	1
Ground substance	Edema	11	4	7
	Stainability	8	0	14
Interepithelial cement		11	0	11
Mucous glands—mucigen granules		10	0	12

Pretreatment biopsies were made on August 24, while posttreatment biopsies were made on September 1, sixteen hours after the last dose of corticotropin. Sections from the biopsies taken before and after treatment were stained simultaneously by the Hotchkiss method and scored as described in Table I. Note that the subepithelial and pericapillary basement membranes of nearly all the treated patients were more prominent after corticotropin.

The nasal mucosa of allergic individuals obtained during the period of hay fever symptoms contains cells not observed in the normal nonallergic mucosa. In addition to the large plasma cells and eosinophils usually found in allergic nasal mucosa, there are pleomorphic, often polygonal multinucleated giant cells whose cytoplasm shows with the Hotchkiss stain many granules of uniform size and staining character (Figs. 3, *B* and 4). No decrease in stainability of these granules occurred in sections that were stained by the Hotchkiss method after being treated with freshly prepared solutions of amylase A and B. This would indicate that the composition of the granules was probably glycoprotein rather than carbohydrate.

Since the granules of eosinophils likewise stained with the Hotchkiss method and since they also frequently contained multiple nuclei, it was necessary to differentiate in sections stained by this method between eosinophils and the multinucleated cells described previously herein. The differentiation was made with some difficulty and in occasional preparations with uncertainty by using adjacent sections cut at 2 microns and stained, respectively, with the Giemsa and Hotchkiss methods. It seemed probable that these cells (Figs. 3, *B*; 4, and 5) were multinucleated, undifferentiated connective tissue cells.

The fibroblasts of the allergic nasal mucosa differ from those of the nonallergic in the staining intensity of their cytoplasm with the Hotchkiss method.

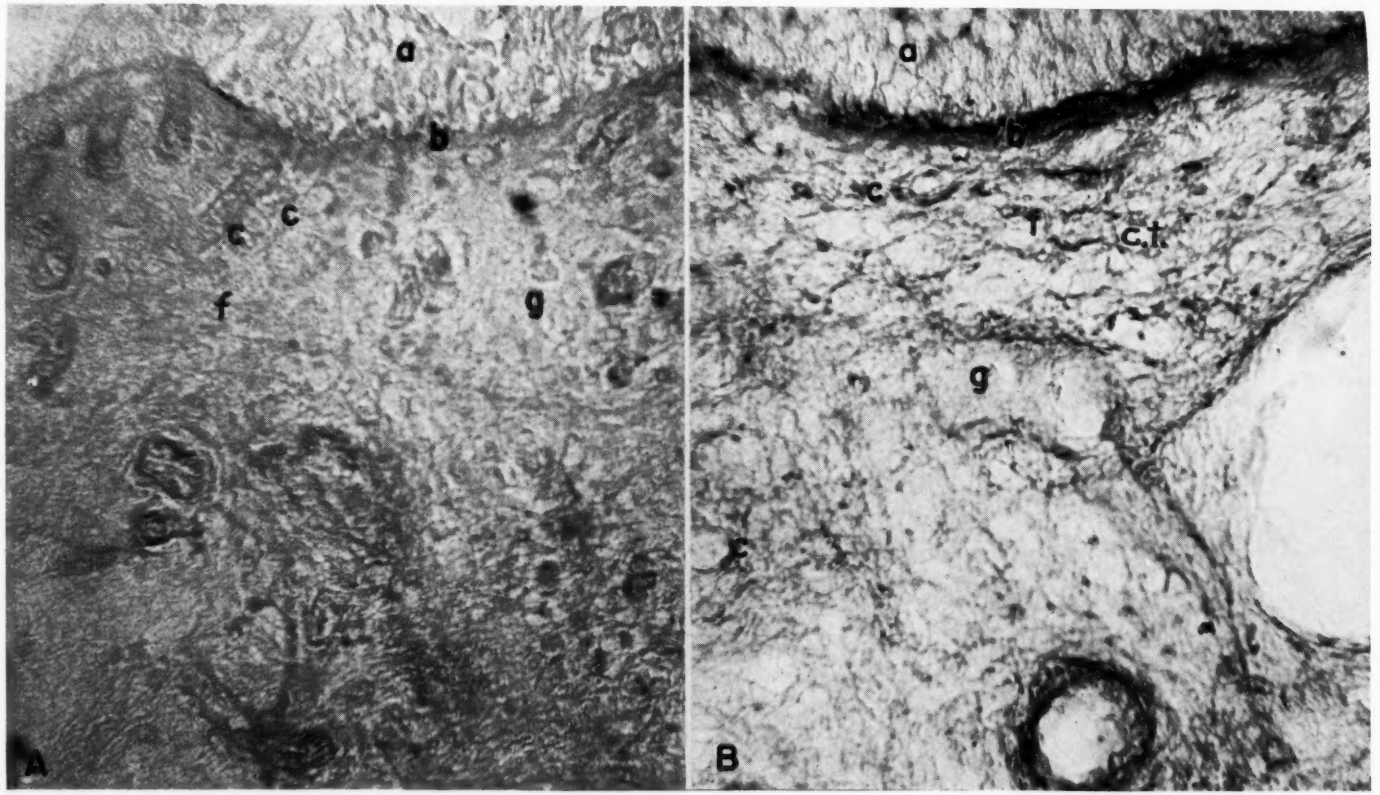

Fig. 2.—*A* and *B* show the nasal mucosa of a patient before and after four days of corticotropin therapy. Note the following histologic changes:

1. The presence of interepithelial cement (*a*) in *B* and its absence in *A*.
2. The wider and deeper stained subepithelial basement membrane (*b*) in *B*, and its representation by a fine, thin wavy line above a faint-staining homogeneous area in *A*.
3. The prominence of the pericapillary basement membrane (*c*), even around the smallest capillaries in *B*, and the difficulty of distinguishing this in *A*.
4. The deeper staining of the ground substance (*g*) in *B*.
5. The increased staining intensity of the fibroblasts (*f*) and of the connective tissue cells (*c.t.*) in *B* and the difficulty of discerning these in *A*.

Sections were cut at 2 microns and stained simultaneously by the Hotchkiss method. The photographs (4 mm. objective: ×450) were developed in the same media for the same period.

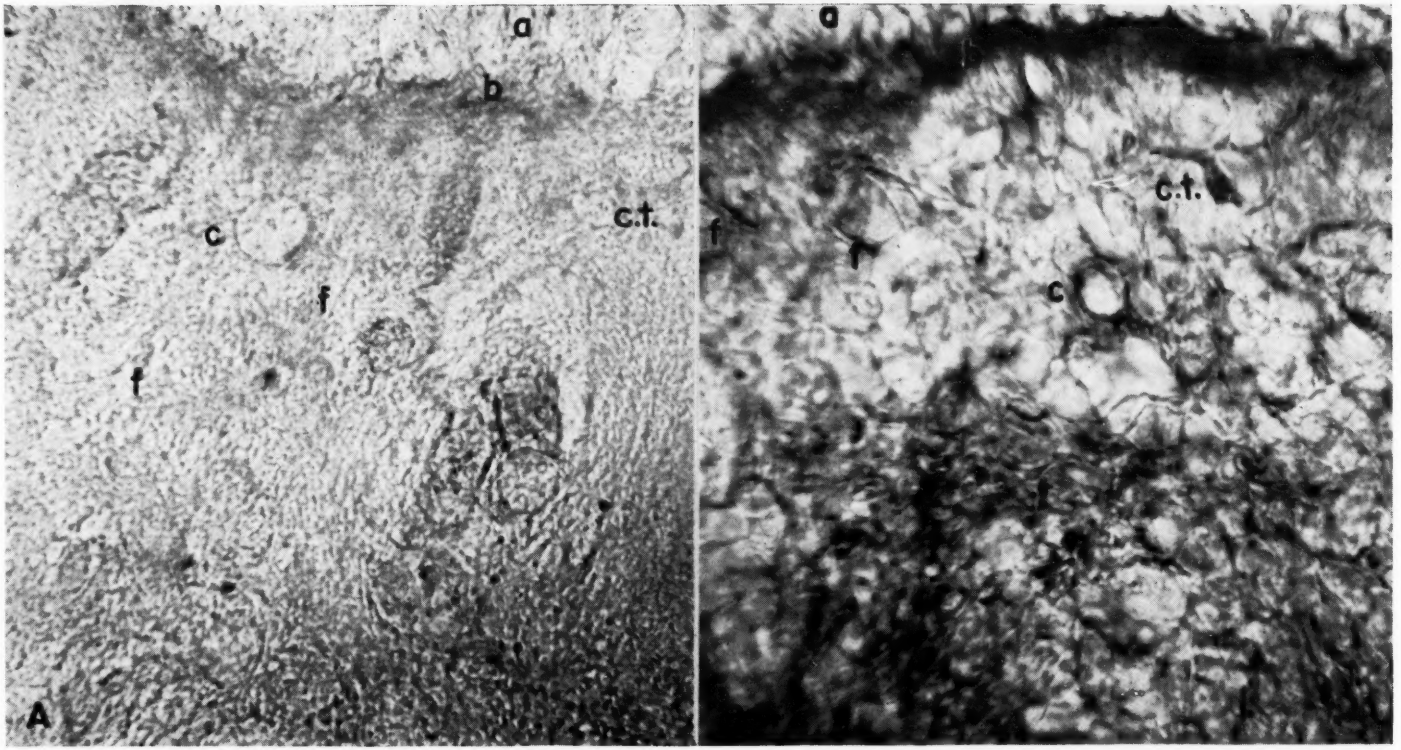

Fig. 3.—A higher magnification of an area in Fig. 2, *A* and *B*. The changes emphasized in *B* as compared with *A* are: the greater amount of intercellular cement (*a*), the greater density and width of subepithelial (*b*) and pericapillary (*c*) basement membranes, and the greater distinctiveness of the fibroblasts (*f*) and connective tissue cells (*c.t.*).

Sections cut at two microns, stained simultaneously by the Hotchkiss method. The photographs (2 mm. oil immersion objective: ×1100) were developed in the same media for the same period.

As allergic symptoms increase, the staining intensity of these cells with the Hotchkiss stain decreases progressively so that, with the increasing duration and intensity of hay fever symptoms, it becomes difficult to distinguish the

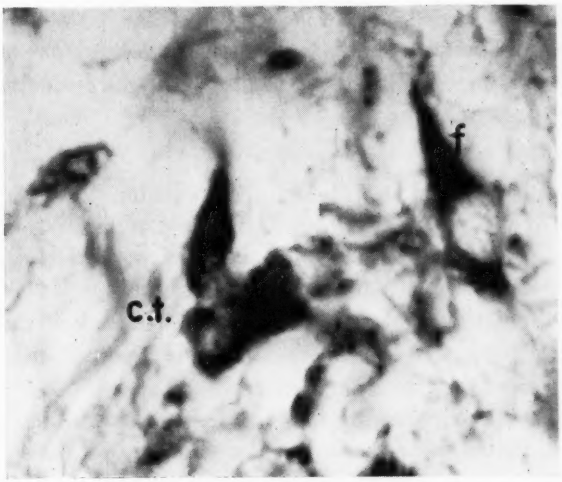

Fig. 4.—A fibroblast (*f*) whose cytoplasm is packed with granules stained by the Hotchkiss method; the multinucleated connective tissue cell (*c.t.*) is described under Fig. 5. Sections were cut at two microns and stained by the Hotchkiss method (×1800).

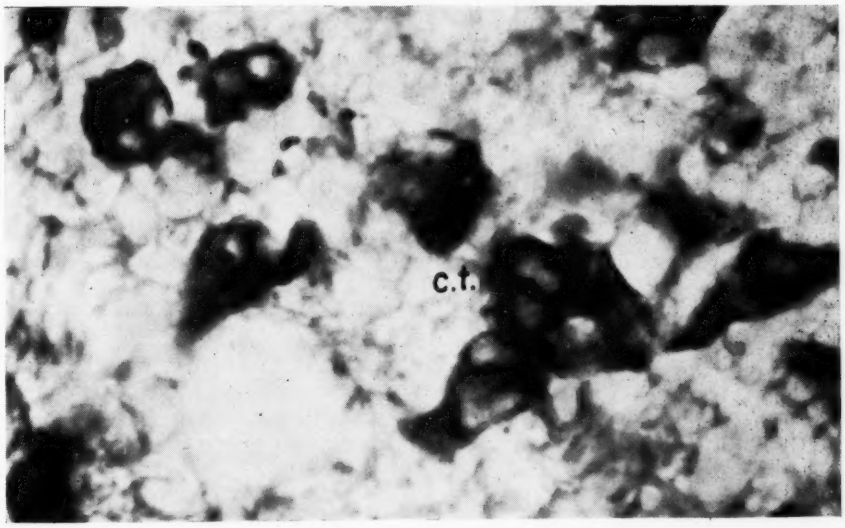

Fig. 5.—Pleomorphic multinucleated cells (*c.t.*) whose cytoplasm stained homogeneously with Giemsa but showed many granules with the Hotchkiss method. These were thought to be mesenchymal connective tissue cells. While present in tissues before treatment, their number as well as staining intensity was increased after corticotropin therapy.
Sections were cut at 2 microns and stained by the Hotchkiss method (×1800).

fibroblasts from the equally pale-staining ground substance (Figs. 2, *A* and 3, *A*). This applies only to allergic patients before treatment with corticotropin.

3. The mucous glands: In addition to a diminution in the Hotchkiss-positive intercellular cement substance, there appeared to be a diminution in the mucigen granules of allergic compared to nonallergic tissues.

C. *Allergic Patients Treated With Corticotropin.*—

Hematoxylin and eosin and Giemsa stains: The tissues of the 1951 group of patients examined with routine staining procedures showed no histologic changes that could be attributed to the effects of corticotropin. This confirms results of a previous study.[3]

Hotchkiss stain:

1. Ground substance: Despite subjective relief and the appearance on gross inspection of decreased swelling of the nasal mucosa following corticotropin therapy, the histologic evidence of edema was sometimes greater in the tissues of the second biopsies of treated patients than in the corresponding tissues removed earlier in the season. In most cases, however, the edema after corticotropin therapy was diminished and the ground substance was more deeply stained.

The basement membranes of the treated patients showed a definite increase in width, density, and affinity for the acid-leukofuchsin stain. The subepithelial basement membrane, as well as those of the capillaries and around the glands, appeared to be restored in a few patients to a status which approached that of the nonallergic nasal tissues (Figs. 2 and 3). The change was best noted by comparing the integrity of these membranes before and after treatment in the 1950 as well as the 1951 group of cases (Tables II and III). Of 22 treated patients in the 1950 group, the width and staining intensity of the subepithelial and pericapillary basement membranes after treatment showed an increase in twenty, a decrease in one, and no change in one (Table II). The ten patients of our 1951 series had similar changes toward normal thickness and staining intensity of the basement membranes following corticotropin therapy (Table III).

TABLE III. COMPARISON OF NASAL BIOPSIES TAKEN FROM 10 RAGWEED-SENSITIVE PATIENTS TREATED WITH CORTICOTROPIN FOR FOUR DAYS DURING AUGUST, 1951

		NUMBER OF PATIENTS SHOWING EFFECTS:		
		INCREASED	DECREASED	UNALTERED
Basement membrane	Subepithelial	8	1	1
	Capillary	8	1	1
Ground substance	Edema	4	4	2
	Stainability	4	4	2
Interepithelial cement		6	3	1
Mucous glands—mucigen granules		7	2	1

Pretreatment biopsies were made on August 24; posttreatment biopsies were made on September 1 within one to three hours after the last dose of corticotropin. Sections from the biopsies taken before and after treatment were stained simultaneously by the Hotchkiss method and scored as described in Table I. Note that the subepithelial and pericapillary basement membranes of nearly all the treated patients were more prominent after corticotropin.

The interepithelial cement showed a less distinct but definite tendency to an increase in stainability in both groups of patients after corticotropin treatment.

2. Cells: The changes produced by treatment with corticotropin in the glycoprotein content of the cytoplasm of fibroblasts were demonstrable with some difficulty because their cytoplasmic granules are barely resolvable under high magnification (×900). Comparison of sections cut at 2 microns from patients before and after treatment revealed that in the 1951-treated group the fibroblasts in 7 out of the 10 posttreatment tissues contained more granules, or stained more deeply by the Hotchkiss method than those in the corresponding pretreatment tissues (Table III, Figs. 2, *A* and *B*, 3, *A* and *B*, and Fig. 4). The cytoplasm of the fibroblasts in two of the remaining three pairs of tissues showed no difference in staining properties, while in the third pair the fibroblasts in the posttreatment tissue showed a decrease in granule content and stainability.

The pleomorphic multinucleated cells with cytoplasmic granules previously described (Figs. 3, *B*, 4, and 5) were present in large numbers (50 to 200 per field of ×500 magnification). Despite the difficulty of differentiating these cells from multinucleated eosinophils in some sections, it was determined that their number, staining intensity, and granule content were definitely increased in tissues after corticotropin treatment as compared with those of corresponding tissues before treatment (Fig. 5; compare also *A* and *B* in Fig. 3).

3. The mucous glands: The number of mucigen granules in the mucous glands of posttreatment tissues showed an increase as compared with the number in the corresponding pretreatment sections. In the 1950 series, 12 patients showed no change following treatment while 10 showed an increase in the number of mucigen granules contained in the posttreatment tissues (Table II). In the 1951 series tissues of seven patients showed an increase, one showed no change, and two showed a decrease in content of mucigen granules in the posttreatment tissues (Table III).

Summary of the histologic changes in the nasal mucosa of allergic patients before and after treatment with corticotropin: The histologic changes that occurred during the period of symptoms in untreated patients were as follows:

1. Edema and decreasing stainability with the Hotchkiss method of the ground substance.

2. Decrease in width and stainability of the basement membranes.

3. Diminished staining intensity of the interepithelial cement.

4. Diminished stainability by the Hotchkiss method of the fibroblasts.

In addition to these changes which were proportional with the severity of symptoms, there was further noted:

5. The presence of a variable number of eosinophils in the tissue.

6. The presence of many round cells resembling plasma cells and of pleomorphic, often polygonal cells with multiple round nuclei. As stained by the Hotchkiss method, the cytoplasm of the latter contained many granules. These latter cells appeared to be an undifferentiated mesenchymal type.

7. A decrease in the content of mucigen granules in the mucous glands.

The histologic changes in the nasal mucosa of allergic patients following corticotropin therapy were as follows:

1. An increase in the width and staining intensity of the basement membranes. This was the most consistent of the changes (Tables II and III and Figs. 2, *B*, and 3, *B*).

2. In patients who showed additional changes there was a tendency to a decrease in the edema and an increase in staining intensity of the ground substance.

3. An increase in the staining intensity of the mature fibroblasts with the Hotchkiss method.

4. The multinucleated pleomorphic cells were increased in number and contained more granules in their cytoplasm.

5. An increase in the number of mucigen granules in the mucous glands.

DISCUSSION

The present study has been mainly concerned with certain aspects of the carbohydrate-containing entities of nasal connective tissue demonstrable by the Hotchkiss technique following fixation by freeze-drying. Tissues were obtained from pollen-sensitive patients during the hay fever season before and after corticotropin therapy, and from normal patients. Interest was focused on:

1. Changes in the ground substance and basement membranes of the epithelium, blood vessels, and glands.

2. Changes in the staining intensity and granule content of the fibroblasts.

In other studies,[8, 10] the concept has been advanced that the ground substance is in a state of constant and, in some cases, rapid change. The basis of these changes is thought to be an alteration in the chemical structure and physical behavior of structural units of connective tissue, having the composition of mucopolysaccharides or glycoproteins. It is supposed that these substances, which are formed by connective tissue cells, are normally aggregated as large molecular units; as such they partake of the nature of high polymers, and confer a relatively stable structure to the tissue. But, under certain conditions, these compounds may be progressively broken down into smaller units. The tissue then becomes less stable or less rigid. This process may be roughly compared with the enzymatic breakdown of a thick, viscid opaque preparation of starch to a water clear solution of maltose; it is essentially one of depolymerization of a complex structure to its individual structural units. Several intermediate stages may be expected, and it is possible to conceive of the process as either partial or complete. In the latter case, a portion of the material might become soluble at some point and enter the blood stream. These different stages of aggregation have been inferred from studies of staining intensity and of solubility of connective tissue in different physiologic states. For example, early stages of connective tissue breakdown may be marked by

an increase in stainability, attributed to swelling and exposure of reactive sites to the Hotchkiss reagent. Later stages in which reactive material has been lost by solution show decreased stainability.

More recent studies[11] have emphasized another aspect of this change. Normal connective tissue of a given site, on the basis of electrochemical measurements, is thought to consist of negatively charged colloid at a given concentration (colloid-rich phase) in equilibrium with tissue fluid (water-rich phase). This two-phase system is of course equilibrated with blood. If from any cause there is a breakdown of one or more component of the colloid-rich phase to give soluble fractions, an influx of dialysate from blood occurs to maintain constant osmotic pressure. This manifests itself as swelling and increased bogginess of the tissue.

In applying these concepts to the present findings, if one assumes that the antigen-antibody reaction in nasal mucosa leads to a shift toward breakdown of connective tissue mucopolysaccharides or glycoproteins, then an explanation is available for some of the observed findings. The general loss in stainability of ground substance and basement membranes could be attributed to the production of soluble residues, which are lost to the circulation. Increased vascular permeability would be due in part to loss in density and thickness of the basement membrane capillaries. Breakdown of colloid determines the shift in balance from a colloid-rich to a water-rich phase, signalized by water uptake and edema.

These changes in the ground substance and basement membranes of the nasal mucosa appear to be important and significant during the allergic reaction. They are not, however, characteristic of allergy only. Some or all of these changes have been observed in such varied situations of connective tissue change as scurvy,[12] inflammation,[8] in the presence of growing tumors,[8, 13] in exfoliative gingivitis,[14] and in ulcerative colitis.[15]

Gersh and Catchpole[8] have suggested that the fibroblasts may secrete material for the formation of ground substance and that the granules observed in their cytoplasm may be precursor material of ground substance. On this basis the following speculations are offered. In nasal allergy there occurs rapid depolymerization of ground substance with uptake of water and electrolytes from the blood to give the typical picture of edema. Concurrently, there may occur removal of soluble material by way of the blood or lymphatic system. Subsequently, fibroblasts are stimulated to increased activity in order to replace the loss of mucoprotein. As the demand increases, undifferentiated mesenchymal cells are mobilized and participate in this function. The demand for newly formed ground substance to replace its constant and rapid loss may finally lead to exhaustion of the capacity both of fibroblasts and immature connective tissue cells to maintain an adequate supply of precursor material. At this stage the fibroblasts have a large amount of cytoplasm which stains faintly with the Hotchkiss method. The mesenchymal cells likewise contain little Hotchkiss positive material in their cytoplasm. Either these lightly stained cells are in a state

of exhaustion, so that little mucoprotein is produced, or the material formed leaves the cell so rapidly that the concentration present at any given time is insufficient to be demonstrable by the Hotchkiss stain.

The effect of corticotropin on the histologic appearance of the nasal mucosa in patients may be briefly summed up as a restoration toward normal of all phases of ground substance and cellular activity. This would imply, in general terms, a termination of the phase of tissue breakdown which we have labeled "depolymerization." The mechanism of this, as of the reverse process, is unknown. It could involve some or all of the following processes:

Inhibition of enzymatic or other processes which favor depolymerization; secretion of normal ground substance by connective tissue cells (assuming a previous secretion of abnormal material); further loss of broken-down moieties, and, with them, loss of fluid. While applied to a very different problem, comparable interpretations were invoked by Aterman[16] to explain the resolution of hepatic fibrosis by cortisone, in so far as deposition and removal of connective tissue were involved.

If one accepts the assumption that the effect of corticotropin is to inhibit the processes which lead to depolymerization, the changes in the ground substance and connective tissue cells may be explained as attempts to restore the normal state. The fibroblasts and mesenchymal cells are enabled to catch up with the production, the delivery, and even the storing of mucoprotein within their cytoplasm. They now stain deeply with the Hotchkiss method and may contain many granules.

In so far as the biologic response during the allergic reaction can be estimated, a quantitative relationship was suggested between the degree of histologic response in the nasal mucosa and the duration of the allergic reaction, or of the pollen dosage, or of a combination of both of these factors. Definitely greater responses were seen in the severe 1951 season than in the 1950 season. In both, tissues removed on September 1 showed greater decrements in staining than those of August 24.

The findings here reported have been confined to the middle turbinates, and changes in this area are believed to be representative of those throughout the nasal mucosa. Findings in tissue from the inferior turbinates removed from a few patients were in no way different. However, the histologic picture presented in this study is incomplete. The eosinophils, for instance, deserve further study. The study of the disruption of these usually hardy cells described in the 1950 study[3] was not pursued further.

SUMMARY AND CONCLUSIONS

Nasal mucosa was obtained by biopsy from normal individuals and from patients sensitive to ragweed pollen before and after corticotropin therapy.

Tissues were fixed by freezing and drying, and were stained routinely and by the periodic acid-fuchsin method for mucopolysaccharides and glycoproteins. Changes involving the connective tissue matrix and connective tissue cells were seen in allergy, and the extent of these changes was related to the severity and duration of exposure. In untreated allergic cases, there

was a decrease in glycoprotein staining of connective tissue cells, of ground substance, and of basement membranes of surface epithelium, capillaries, and glands; interepithelial cement of surface and gland cells was diminished, and mucigen granules of glands were decreased. Corticotropin reversed these changes.

The authors wish to acknowledge the assistance of Dr. Isadore Gersh, Professor of Anatomy, University of Chicago.

The technical assistance of Catherine Bader, B.A., is gladly acknowledged.

We are indebted to Dr. David Klein, Director of Wilson Laboratories, for the generous supply of corticotropin used in this study.

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INHIBITION OF HISTAMINE DEATH IN PERTUSSIS-INOCULATED MICE BY CORTISONE AND NEOANTERGAN

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THE guinea pig is naturally sensitive to histamine; for this reason, it has been widely employed in the screening of compounds which inhibit the effects of this toxic amine. The mouse is naturally resistant to histamine; however, Parfentjev and Goodline[1] have shown that previous inoculation of mice with *Hemophilus pertussis* vaccine greatly increases the sensitivity of these animals to histamine. In an experiment performed by the author,[2] the LD_{50} of histamine in uninoculated mice was found to be 2300 mg./kg. whereas the LD_{50} of histamine in pertussis-inoculated mice was only 23 mg./kg. This report demonstrates that the antihistamine, Neoantergan, and the adrenal steroid 11-dehydro-17-hydroxy corticosterone (Cortone, Merck), protected pertussis-inoculated, histamine-sensitive mice from the lethal effects of histamine. Histamine death in guinea pigs has been inhibited by Neoantergan[3]; however, cortisone and ACTH have not been effective.[4, 5, 6] The inhibition of histamine death in pertussis-inoculated mice with antihistamines has been reported by Parfentjev and Goodline[1] and by Parfentjev.[7] The antihistamines employed were Bromothen and Tephorin.

Some of the following experiments also show that cortisone protected uninoculated mice, as well as mice previously inoculated with *H. pertussis* vaccine, from the lethal effects of subsequent injections of *H. pertussis* vaccine.

MATERIALS AND METHODS

H. pertussis Vaccine.—Phase I *H. pertussis* vaccine 80855A* (90 billion organisms per c.c.) consisted of washed bacterial cells which had been grown on sheep blood agar. It was prepared from a pool of 6 different strains of organisms and was preserved with phenol.

Mice.—White, 20 to 40 gram, male and female mice were employed. Some were obtained from the departmental mouse colony, some were purchased from dealers, but the great majority were received from Dr. J. L. Melnick of the Section of Preventive Medicine, Yale University. Mice from this latter source had previously been inoculated intracerebrally with either Lansing poliomyelitis virus and antiserum, or West Nile virus and antiserum, and had survived. Parallel experiments employing such postneutralization test (PNT), mice and normal mice revealed no significant differences in their response to

This paper is part of a dissertation presented to the faculty of the graduate school of Yale University in candidacy for the degree of Doctor of Philosophy.

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Received for publication, July 28, 1952.

*Supplied through the courtesy of the E. R. Squibb Co.

various materials following pertussis inoculation. In most studies, two or more groups of mice were used. Randomization of the animals was accomplished by the use of a die or by a table of random numbers.

Histamine.—Histamine diphosphate (Eimer and Amend) was used throughout these experiments. In most cases, 1 per cent solutions were used; however, when large doses of histamine were required, 10 per cent solutions were utilized. Histamine diphosphate was dissolved in distilled water and the solution was then sterilized by passing it through an L_5 Chamberland filter. Histamine was injected on the basis of milligrams of histamine diphosphate per kilogram of mouse weight. The total volume inoculated varied from 0.05 c.c., to 0.6 c.c. An experiment was performed which indicated that volume differences of this order did not influence the toxic effects of histamine.

Sensitizing Procedure.—A modification of the method employed by Parfentjev and Goodline[1] was employed. Mice were sensitized by intraperitoneal injections of *H. pertussis* vaccine. In order to reduce the number of toxic deaths during the sensitization procedure, one-third of the sensitizing dose was inoculated on each of three successive days. Six days after the last sensitizing injection, mice were challenged intraperitoneally with histamine, *H. pertussis* vaccine, or other material. Deaths occurring within a 24-hour period were noted, previous experience having indicated this to be an adequate period.

EXPERIMENTAL

Inhibition of Histamine Death by Cortisone.—Twenty to 30 gram male and female, white mice were sensitized with 0.2 c.c. of *H. pertussis* vaccine 80855A on each of three successive days. Six days after the last sensitizing injection, all animals were challenged with 25 mg./kg. of histamine. Eighteen hours before the challenge dose of histamine was administered the mice were divided into two groups. Test animals received 3 mg. of cortisone intramuscularly; control animals were injected with an equal volume of saline.

The data in Table I indicate that only 13 per cent of the cortisone-treated mice died from an intraperitoneal injection of histamine which was fatal to 58 per cent of the untreated control animals. The difference between the percentage of deaths in the two groups of animals is a significant one ($p = < .01$). It was later determined that as little as 0.6 mg. of cortisone would protect sensitized mice from an LD_{50} of histamine.

Since cortisone contains 1.5 per cent benzyl alcohol as preservative, and sodium carboxymethylcellulose, sodium chloride, and polyoxyethylene sorbi-

TABLE I. INHIBITION OF HISTAMINE DEATH IN SENSITIZED* MICE BY CORTISONE

	CHALLENGE DOSE OF HISTAMINE† (MG./KG.)	SURVIVORS	DEATHS	TOTAL	PER CENT OF DEATHS
Cortisone-treated mice	25	47	7	54	13
Control mice	25	21	29	50	58

$p = < .01$

*Sensitized with 0.2 c.c. of *H. pertussis* vaccine 80855A on each of three successive days.

†6 of 6 uninoculated mice survived this dose of histamine.

tan mono-oleate as suspending agents, these materials were tested for their protective effects against histamine. It was found that these preservatives and suspending agents possessed no inhibitory activity against the lethal effects of histamine.

Protection of Pertussis-Inoculated Mice from the Lethal Effects of H. pertussis Vaccine.—Parfentjev and his co-workers have shown that mice inoculated with *H. pertussis* vaccine become sensitive not only to histamine[1] but to subsequent injections of pertussis nucleoprotein and pertussis vaccine as well.[8] The following experiment demonstrates that cortisone protected a significant percentage of pertussis-inoculated mice from death due to subsequent injections of pertussis vaccine.

Twenty-five to 35 gram PNT mice were injected with 0.2 c.c. of *H. pertussis* vaccine 80855A on each of three successive days. The mice were then divided into four groups. Eighteen hours before a challenge dose of 0.1 c.c. of *H. pertussis* vaccine, Groups I to III received intramuscular injections of the following amounts of cortisone: Group I, 0.3 mg.; Group II, 1 mg.; Group III, 3 mg. The total volume injected was 0.2 c.c. Group IV was inoculated with this amount of saline. The challenge dose of pertussis vaccine was administered on the sixth day after the last sensitizing injection.

The data in Table II indicate that 0.1 c.c. of *H. pertussis* vaccine killed 74 per cent of the untreated animals, 75 per cent of the mice given 0.3 mg. of cortisone, 43 per cent of the animals injected with 1 mg. of cortisone, and only 4 per cent of the mice inoculated with 3 mg. of this adrenal steroid. Calculations based on the data in Table II showed that 1 mg. of cortisone protected a significant percentage of pertussis-inoculated mice from death due to subsequent injections of pertussis vaccine.

TABLE II. EFFECT OF DOSAGE OF CORTISONE ON ITS PROTECTIVE ACTION AGAINST LETHAL EFFECTS OF PERTUSSIS VACCINE* IN SENSITIZED MICE

	AMOUNT OF CORTISONE	SURVIVORS	DEATHS	TOTAL	PER CENT OF DEATHS
Test Mice I	0.3 mg.	5	15	20	75
Test mice II†	1.0 mg.	13	10	23	43
Test mice III‡	3.0 mg.	22	1	23	4
Control mice IV		7	20	27	74

*6 of 6 uninoculated mice survived 0.1 c.c. of pertussis vaccine.

†The difference between the percentage of deaths in Group II and the Control Group IV is a significant one ($p = .01$).

‡The difference between the percentage of deaths in Group III and the Control Group IV is a significant one ($p = < .01$).

It was of interest to determine whether cortisone would also inhibit the lethal effects of *H. pertussis* vaccine in uninoculated mice. If cortisone could be shown to protect uninoculated mice from toxic death due to *H. pertussis* vaccine, it would not be necessary to consider its prevention of death due to pertussis vaccine in mice previously injected with this vaccine on the basis of some antiallergic property it might possess.

The data listed in Table III demonstrate that 62 per cent of the untreated control animals were killed by an intraperitoneal injection of 1 c.c. of *H. per-*

tussis vaccine; in contrast, only 7 per cent of the cortisone-treated animals succumbed to this procedure. The difference between the two groups is a significant one ($p = .01$). Cortisone, therefore, protected uninoculated mice from toxic death due to *H. pertussis* vaccine.

TABLE III. PROTECTION OF NORMAL MICE WITH CORTISONE FROM TOXIC DEATH DUE TO H. PERTUSSIS VACCINE

	SURVIVORS	DEATHS	TOTAL	PER CENT OF DEATHS
Cortisone-treated mice	15	1	16	7*
Control mice	6	10	16	62

*The difference between the percentage of deaths in the cortisone-treated mice and in the control mice is a significant one ($p = .01$).

The Effect of the Antihistamine Neoantergan Against Histamine and H. pertussis Vaccine.—Twenty to 35 gram PNT male and female, white mice were injected with 0.2 c.c. of *H. pertussis* vaccine 80855A on each of three successive days. Six days after the last sensitizing injection, the animals were divided into four groups which were inoculated in the following manner: Group I received 50 mg./kg. of the antihistamine, Neoantergan intraperitoneally, followed within 10 to 20 minutes by 25 mg./kg. of histamine; Group II was injected with distilled water (equivalent in volume to the Neoantergan solution used in Group I); followed within 10 to 20 minutes by 25 mg./kg. of histamine; Group III received 50 mg./kg. of Neoantergan followed within 10 to 20 minutes by 0.1 c.c. of *H. pertussis* vaccine 80855A; Group IV was inoculated with distilled water followed in 10 to 20 minutes by 0.1 c.c. of *H. pertussis* vaccine 80855A.

TABLE IV. NEOANTERGAN AGAINST THE LETHAL EFFECTS OF HISTAMINE AND PERTUSSIS VACCINE IN PERTUSSIS-INOCULATED MICE

GROUP NO.	MICE SENSITIZED WITH:	INJECTED 6 DAYS LATER WITH:	SURVIVORS	DEATHS	TOTAL	PER CENT OF DEATHS
I	0.2 c.c. of *H. pertussis* vaccine 80855A on each of 3 successive days	50 mg./kg. of Neoantergan followed by 25 mg./kg. histamine*	20	0	20	0
II	0.2 c.c. of *H. pertussis* vaccine 80855A on each of 3 successive days	Distilled water followed by 25 mg./kg. of histamine	13	17	30	56
III	0.2 c.c. of *H. pertussis* vaccine 80855A on each of 3 successive days	50 mg./kg. of Neoantergan followed by 0.1 c.c. of *H. pertussis* vaccine	10	12	22	55
IV	0.2 c.c. of *H. pertussis* vaccine 80855A on each of 3 successive days	Distilled water followed by 0.1 c.c. of *H. pertussis* vaccine†	11	8	19	41

*6 of 6 uninoculated mice survived this dose of histamine.
†6 of 6 uninoculated mice survived this dose of H. pertussis vaccine.

The data summarized in Table IV show that 25 mg./kg. of histamine killed 56 per cent of the unprotected animals; however, no mice died from this dose

of histamine when it was preceded by 50 mg./kg. of Neoantergan. The difference between the percentage of deaths in these groups is a significant one ($p = < .01$).

One tenth of a c.c. of *H. pertussis* vaccine was fatal to 41 per cent of unprotected mice; it was also lethal to 55 per cent of the animals given Neoantergan prior to the challenge dose of pertussis vaccine. The difference between the percentage of deaths in these latter groups is not significant.

It is apparent, therefore, that the antihistamine, Neoantergan, protected pertussis-sensitized mice from the lethal effects of histamine, but not from fatal amounts of *H. pertussis* vaccine.

It should be noted, however, that histamine kills sensitized mice within an hour, whereas the majority of sensitized mice which succumb to injections of pertussis vaccine die in 18 to 24 hours.

Effect of the Dosage of Neoantergan on Its Protective Action Against Histamine.—Twenty to 35 gram PNT male and female, white mice were inoculated with 0.1 c.c. of *H. pertussis* vaccine 80855A on each of three successive days. On the sixth day after the last inoculation, the animals were divided into four groups. Group I was injected with 1.0 mg./kg. of Neoantergan; Group II, 3 mg./kg. of this antihistamine; Group III was inoculated with 10 mg./kg. of Neoantergan; Group IV received distilled water in amounts equivalent to the volumes of antihistamine administered to Group III. In each batch of animals, these injections were followed within 10 to 20 minutes by a challenge dose of 25 mg./kg. of histamine.

TABLE V. EFFECT OF THE DOSAGE OF NEOANTERGAN ON ITS PROTECTIVE ACTION AGAINST HISTAMINE*

	AMOUNT OF NEOANTERGAN	SURVIVORS	DEATHS	TOTAL	PER CENT OF DEATHS
Test mice I	1 mg./kg.	7	8	15	53
Test mice II†	3 mg./kg.	14	2	16	13
Test mice III‡	10 mg./kg.	15	0	15	0
Control mice IV	—	4	11	15	73

*The dose of histamine employed (25 mg./kg.) was not lethal to 6 of 6 uninoculated mice.

†The difference between the percentage of deaths in Group II and the Control Group IV is a significant one ($p = < .01$).

‡The difference between the percentage of deaths in Group III and the Control Group IV is a significant one ($p = < .01$).

Calculations based on the data compiled in Table V indicate that as little as 3 mg./kg. of Neoantergan protected a significant percentage of sensitized mice from a dose of histamine which was fatal to 73 per cent of the control animals. Rose and his associates[3] have reported that 3 mg./kg. of Neoantergan protected 16 of 16 guinea pigs from an LD_{100} of histamine (0.4 mg. of histamine base per kg.).

DISCUSSION

Cortisone has been shown to be effective in various allergic conditions. Clinically, the administration of this hormone has been beneficial in asthma, hay fever, urticaria, and various types of eczema. In experimental hyper-

sensitivity of animals, the use of cortisone or ACTH has produced the following results: (1) Inhibition of the tuberculin skin reaction and tuberculin shock in sensitized guinea pigs.[9] (2) Suppression of the Shwartzman phenomenon.[10] (3) Prevention of the development of periarteritis nodosa and cardiac inflammation in rabbits made sensitive to horse serum.[11] (4) Inhibition of the development of the Arthus state.[12] (5) Inhibition of anaphylaxis in the mouse.[13]

Fischel has studied the effect of adrenal cortical hormones in relation to several steps in the development of immune or allergic reactions.[14] He found that anaphylaxis, the local passive Arthus reaction, and other manifestations of the union of antigen with antibody were not appreciably altered. However, an inhibition of antibody production was produced by ACTH.

The reduction of circulating antibodies by ACTH and cortisone may not be the only explanation of the striking effect of these hormones in allergic conditions. Since allergic and anaphylactic reactions seem to be produced by histamine, histamine-like substances, or other metabolites, the effect of cortisone upon the lethal action of histamine in pertussis-inoculated mice was studied.

The data in Table I indicate that cortisone inhibited the fatal effects of histamine in pertussis-inoculated mice. However, cortisone is not a specific histamine antagonist.[15] Inflammatory reactions induced by such diverse stimuli as burns, turpentine, glycerol, tubercle bacilli, trauma, mustard oil, and formaldehyde have been attenuated or inhibited by the action of this hormone.[16] Factors in the inflammatory response reported to be affected by ACTH and cortisone were vascular changes, leukocytic infiltration, connective tissue formation, and macrophage accumulation.[16] Cortisone thus seems to act by altering the capacity of the host's tissue to respond to various types of irritants. Its inhibitory action in hypersensitive states might therefore be directed against the inflammatory response induced by the type of irritants peculiar to these conditions, namely, histamine or other substances released from cells as a consequence of an antigen-antibody reaction.

In contrast to the failure of cortisone or ACTH to prevent histamine death in the guinea pig, experiments reported in this paper have indicated that cortisone could inhibit the fatal effects of histamine in mice previously inoculated with *H. pertussis* vaccine. This latter finding makes untenable the view[16] that allergic reactions inhibited by cortisone do not involve histamine and that allergic reactions not affected by cortisone are due to histamine.

A possible explanation for the failure of cortisone to prevent histamine death in the guinea pig might lie in the extreme sensitivity of this animal to histamine (LD_{50} of 0.2 to 0.4 mg./kg. of histamine base) and in the failure of various investigators to challenge the pigs with an LD_{50} of histamine (admittedly a difficult procedure in this animal). In many cases, guinea pigs have been injected intravenously with an MLD of histamine and death occurred almost immediately. In contrast, sensitized mice were challenged intraperitoneally by the author with an LD_{50} of histamine. Of the unprotected control

mice which succumbed to this dose of histamine, a majority expired in thirty minutes; the remainder died within 24 hours. Wilson, Booth, and DeEds[17] have reported that flavonoids protected guinea pigs against an LD_{50} of histamine but not against an LD_{100} of this toxic amine.

It has been shown that the adrenal steroid, cortisone, and the antihistaminic, Neoantergan, protected sensitized mice from histamine death. It is quite likely that mice inoculated with *H. pertussis* vaccine, and thus rendered sensitive to the lethal effects of histamine, could be used to screen other types of compounds for their activity against histamine. The guinea pig has been widely employed for this purpose. However, the use of mice previously injected with *H. pertussis* vaccine would seem to be more advantageous for the following reasons:

1. Numerous experiments have indicated that it is an easy matter to determine the LD_{50} of histamine in mice previously inoculated with *H. pertussis* vaccine; mice from three different sources have been used and at least 5 different commercial Phase I *H. pertussis* vaccines have been successfully employed. It is much more difficult to obtain an LD_{50} of histamine in guinea pigs. Wilson, Booth, and DeEds[17] reported that a 5 per cent change in dosage was sufficient to change an LD_{50} to an LD_{100} or an LD_0. They also stated that sensitivity to histamine varied from group to group of animals; in cases where the protective action of a compound was slight, negative results were obtained when the amount of histamine employed killed all or nearly all of the control animals.

2. Mice are much less expensive than guinea pigs, larger numbers of animals can be used, and results are easier to evaluate.

3. The sensitivity of normal mice to histamine is greatly increased after pertussis inoculation. The normal guinea pig, however, is naturally sensitive to histamine and there is no evidence that a guinea pig sensitized to various antigens becomes even more sensitive to this agent.[18] The vital capacity of asthmatic individuals is lowered by inhalation of amounts of histamine which do not affect normal people.[19] It is possible, therefore, that the increased sensitivity of mice to histamine after they have been inoculated with *H. pertussis* vaccine is more analogous to human allergy than is histamine sensitivity in the guinea pig.

SUMMARY

Cortisone inhibited the lethal effects of both histamine and *H. pertussis* vaccine in pertussis-inoculated mice. Cortisone also protected uninoculated mice from toxic death due to *H. pertussis* vaccine.

The antihistamine, Neoantergan, protected pertussis-sensitized mice from the lethal effects of histamine but not from fatal amounts of *H. pertussis* vaccine.

The advantages of using pertussis-inoculated, histamine-sensitive mice rather than guinea pigs in the screening of compounds which might be active against histamine are discussed.

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EPINEPHRINE BASE SUSPENDED IN WATER WITH THIOGLYCOLATE

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THE effectiveness of many therapeutic agents has been increased by prolonging their absorption after injection. A method commonly used for this purpose depends on the conversion of the agents to insoluble suspensions, such as protamine zinc insulin, and procaine penicillin. Epinephrine base suspended in oil[1] has been used for prolonged effect. However, it has the disadvantages that because of its delayed action it can not be used when a prompt epinephrine effect is desired, e.g., an acute asthmatic attack, and it must be given intramuscularly which makes self-administration by the patient difficult. Aqueous suspensions of slightly soluble epinephrine base have a prompt, as well as a prolonged action, and may be self-administered subcutaneously as readily as epinephrine hydrochloride solution.

The original method for preparing aqueous epinephrine suspension consisted of adding epinephrine base to a water solution of sodium thioglycolate.[2] Since then, finer suspensions have been prepared by precipitating epinephrine base from a solution of epinephrine thioglycolate. The purpose of this paper is to describe this new method, and to discuss the clinical use of such suspensions and their advantages.

MATERIALS AND METHOD

Synthetic epinephrine base* is dissolved in thioglycolic acid† in proportion of 1 Gm. of base to 1 ml. of acid. Glycerin U.S.P. is then added to make a 10 per cent concentration of base. For sterility, the solution is autoclaved, or sufficient 88 per cent phenol is added to make a 2 per cent concentration. The glycerine solution (Solution A) is placed in a sterile rubber capped bottle until used.

Solution B is 1.5 normal sodium hydroxide in a 20 per cent water and 80 per cent glycerine mixture, which may be autoclaved.

To make 1,000 ml. of an epinephrine suspension containing 5 mg. per ml., 50 ml. of Solution A are mixed with 50 ml. of Solution B, and the mixture shaken until precipitation occurs. When diluted, this mixture will have a pH of 8.0 ± 0.5. If necessary, the pH may be brought to the indicated range by the use of sodium hydroxide or hydrochloric acid as needed. The amount of glycerine present in the mixture is 90 ml., considering 50 ml. in Solution A, and 40 ml. in Solution B. As 250 ml. of glycerine is required to make a final 25 per cent concentration, 160 ml. of glycerin must be added. Phenol in final concentration of 0.44 per cent is used as a preservative. If Solution A was made with phenol,

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Received for publication Feb. 19, 1952.
*Kindly supplied by Burroughs Wellcome & Co., Inc.
†Practical Grade, obtained from the Eastman Kodak Co.

4 ml. of 88 per cent phenol must be added; if Solution A was not made with phenol, 5 ml. must be added. Distilled water sufficient to make a final volume of 900 ml. is mixed with the additional glycerine and phenol required, autoclaved, and added to the 100 ml. mixture of Solution A and B.

The final composition is as follows:

Epinephrine	0.5% (1-200)
Thioglycollate	0.5%
Glycerine	25.0%
Phenol	0.44%

An epinephrine suspension made according to the above method, is now generally available as Sus-phrine (Brewer).

DISCUSSION

When Solution A and Solution B made in water, in the same proportions but without glycerine, are mixed, precipitation is slow and coarse. If the water solutions are mixed with a magnetic stirrer, precipitation is prompt and finely divided. The same finely divided precipitate is obtained when Solution A and Solution B, made in glycerin as described above, are simply mixed. The glycerin evidently prevents the conglomeration of the precipitated particles. The final concentration of glycerin is made 25 per cent to provide a viscosity sufficient to slow settling of the particles and to facilitate withdrawal of aliquot proportions from the dispensing vial. This concentration of glycerin is well tolerated by the subcutaneous tissues.

A final 0.5 per cent concentration of epinephrine (5 mg./ml.) has been selected because, from clinical experience, patients can tolerate the same volume of this 1-200 suspension as they can of the standard epinephrine hydrochloride 1-1,000 solution, although receiving five times as much epinephrine when the suspension is used.

A thioglycolate concentration up to 2.0 per cent has been tried clinically. Such concentrations are apt to be painful and may result in purplish discoloration of the skin at the site of injection with necrosis and blister formation, possibly from local anoxia. The 0.5 per cent concentration is well tolerated, and prevents oxidation of the suspensions in filled, capped vials. Frequently, after a vial has been used, the last few milliliters turn brownish. A slight discoloration with persistence of precipitate seems to make no difference in the therapeutic efficacy. When the preparation turns dark brown, some or all of the suspension may dissolve with varying loss of activity, and the preparation should be discarded.

Thioglycolic acid was selected as the reducing agent because experience with sodium thioglycolate culture media has demonstrated its effective reducing action, and its tolerance by living bacterial cells. The reducing action is dependent on the -SH group in its molecule. Other organic compounds with the -SH group might also be effective.

Preparations varying in pH from 7.5 to 9.0 have been used with no apparent difference in efficacy. A pH of about 8.0 has been selected arbitrarily. A phosphate buffer at that pH might be helpful in the final preparation.

In the final preparation, about 25 per cent of the epinephrine is present in solution in the supernatant.* The prompt epinephrine effect after injection is probably due to the dissolved fraction, and the prolonged effect to the precipitated fraction.

CLINICAL USE

Dosage: The dose of epinephrine hydrochloride U.S.P. 1-1,000 is usually given as 0.3 ml. to 0.5 ml. In many patients, 0.5 ml. produces distressing side effects. Most patients get satisfactory relief from doses of 0.1 ml. to 0.3 ml., and some can not tolerate more than 0.1 ml. In general, patients tolerate the same volume of the 1-200 suspension as the 1-1,000 solution, and are less inclined to have immediate side effects. A safe starting dose of the suspension is, therefore, 0.1 ml. to 0.3 ml. decreasing if necessary, or increasing to 0.5 ml. as tolerated and necessary. When using epinephrine hydrochloride 1-1,000 solution, the smallest amount capable of providing relief is indicated since larger doses do not give proportionately longer relief. When using suspensions, the largest amount which can be tolerated is indicated in order to provide as large an effective depot as possible. One 70-year-old man with chronic bronchial asthma, has increased his dosage to 0.65 ml. However, doses over 0.5 ml. should be approached cautiously and only when necessary.

In most instances one or two doses a day are sufficient. During periods of exacerbation of symptoms, three or four doses a day may be necessary. In acute situations, the initial dose selected may be insufficient, and another injection may be required after fifteen or thirty minutes.

Administration and Local Reactions.—After the vial is shaken to disperse the precipitate, the desired dose is withdrawn and promptly injected subcutaneously with the usual hypodermic needle. A central area of blanching appears in a few minutes, which gradually increases and may persist for 10 hours or more. An area of redness surrounds the blanched area. The reaction disappears with no residual, except for slight tenderness. When injections are given frequently in the same area, induration of the skin may develop, which quickly subsides when the area is avoided. No persistent changes have been encountered after use over periods as long as ten years.

General Reactions.—Rarely, severe general reactions with apprehension, headache, pallor, palpitation, and nausea, lasting up to 10 minutes, have been reported by two patients following self-administered injections. No such reactions have been observed following injections by physicians or nurses. Both these patients have continued to use the suspension. One patient, a 36-year-old woman with chronic asthma who has taken one or two doses of 0.30 ml. daily for three years, has had two reactions. The other patient, a 70-year-old man with bronchial asthma, has taken doses of 0.65 ml. irregularly from twice daily to twice weekly for three years and has had about three reactions.

Clinical Indications.—In general, epinephrine suspension has the same indications as epinephrine solution 1-1,000, and epinephrine in oil. Its action is about as prompt as the solution, manifested in 1 to 5 minutes, and as prolonged

*Determinations made by Brewer & Co.

as the oil suspension, lasting 8 to 12 hours. Patients with chronic bronchial asthma requiring one or more injections of epinephrine 1-1,000 daily for relief, form the group most benefited by the use of epinephrine suspension in water The injections may be taken when attacks begin, or may be taken in the morning and evening prophylactically. When necessary the usual antiasthmatic medications may be used in addition if mild symptoms appear between injections. A case example follows.

CASE REPORTS

A. R., a 47-year-old theater manager, had bronchial asthma since childhood, aggravated by pollen, infection, and emotional factors. When seen in 1942, he was taking ten to twelve injections of epinephrine 1-1,000 daily, in doses of 0.5 ml. to 0.75 ml. Since February, 1942, he has taken epinephrine suspensions daily, at first four or five injections a day and later two or three. For the past two years, with the oral use of Fowler's Solution, he has needed one or two injections daily.

Patients who are awakened at night by wheezing, even if attacks are mild enough to be relieved by simple measures, may be benefited by an injection of epinephrine suspension at bedtime in order to provide an uninterrupted sleep. A case example follows.

S. M., a 36-year-old housewife, has had hay fever and asthma since childhood. In December, 1948, she was admitted to the Beth Israel Hospital for severe asthma, which was unrelieved by epinephrine 1-1,000 in doses which caused palpitation and distress. Epinephrine suspension, three times a day, gave marked relief. Since then she has taken one injection at bedtime and sleeps through the night. During the day she takes oral and nebulized medication as needed. Occasionally a second injection is taken in the morning when she feels that oral medication would not be adequate.

For isolated attacks of bronchial asthma, acute constitutional reactions following injections of antigens, severe urticaria and angio-neurotic edema, conditions usually requiring injections of epinephrine, the suspension is preferable because of its prolonged effect as well as its prompt action.

Frequent injections of epinephrine solution are sometimes needed in cases of Stokes-Adams syndrome. Epinephrine suspensions may be tried in such cases.

Patients in status asthmaticus, resistant to epinephrine solution 1-1,000 may not be expected to respond to epinephrine suspension. Epinephrine suspension should not be given intravenously.

SUMMARY

1. A new method of making an epinephrine base suspension in water is described.

2. In clinical use, this epinephrine suspension produces a therapeutic effect as prompt as that of solutions of epinephrine and as prolonged as that of suspensions of epinephrine in oil.

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STUDIES IN FOOD SENSITIVITY

II. The Effect of Protein Digestion on Antigenicity of Foods as Determined by Skin Tests and Clinical Food Trials

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IN THE diagnosis and treatment of allergic diseases, skin testing has been an important step in the attempts to determine the causative agents of any particular allergic disorder. In general, skin tests with pollens and inhalants, when properly performed and interpreted, have yielded sufficiently reliable information to be of diagnostic value. It is in skin testing with foods that we have encountered a stumbling block in our diagnostic approach to the etiology of allergic diseases. Here one finds so many inconsistencies in attempting to correlate the results of skin tests with the clinical symptoms that many allergists have come to the conclusion that skin testing with food extracts is of little or no value.

In 1923, Wacker, Wetmore, and Adkinson[1] studied the reactions in skin tests of artificial digests of food proteins as compared to standard extracts. They found that, with the exception of two cases, all cases that showed negative skin tests to the whole protein showed negative skin tests to the digests. From these observations they reached the conclusion that digestion products of proteins did not sensitize human beings and did not play a part in human allergy, and that, where sensitization took place, it was probably due to the assimilation into the blood stream of small quantities of unaltered protein.

However, as time went on, it became evident that positive skin tests could be obtained with substances other than unaltered proteins. In 1939 Winkenwerder, Buell and Howard[2] tested a group of ragweed cases with dilute solutions of nucleic acid, and in 50 cases obtained immediate wheal and erythema reactions. In ten of these patients, passive transfer reactions to nucleic acid were positive and in two of the ragweed patients, tested with nucleic acid, severe constitutional reactions developed.

Further studies along this line were made by Cooke and his co-workers[3] when they observed a fairly severe reaction occurring promptly in a patient after the administration of a second injection of tetanus toxoid. They found the reaction to be due to supposedly nonantigenic proteose, which was used in most tetanus culture media and which was present in tetanus toxoid. Subsequent studies with proteoses, from known sources, showed definite specific antigenicity in the guinea pig for primary proteoses of milk, whey, casein, beef, and chicken.[4]

These reports were followed by other studies regarding the significance of such findings as related to food sensitivities in human beings. It was assumed that, since the products of early protein digestion (primary proteoses) were antigenic, it might be possible to explain, as due to products of food digestion, certain clinical reactions which followed the ingestion of foods but in which

Received for publication, April 7, 1952.
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skin tests to the original unaltered protein of such foods were negative. Using solutions of primary and secondary proteoses prepared from known sources, Cooke[5] tested a series of 39 allergic patients. In five patients he reported positive reactions to skin tests and passive transfer tests with these substances, whereas the standard extracts of these foods gave negative skin tests. These patients were all clinically sensitive to the foods in question. Cooke, therefore, arrived at the conclusion that clinical sensitization to protein digestion derivatives, at least at the proteose stage, does exist; and that, in some cases of food allergy, delayed clinical reactions can be explained on this basis. In such cases, skin tests with extracts of suitable preparations of artificially digested proteins give positive reactions.

Cooke's thesis was further substantiated by Blamoutier,[6] who studied a case of urticaria in which symptoms occurred approximately five to six hours after the ingestion of lamb, and lasted from twenty-four to thirty-six hours. Cutaneous (scratch) tests with standard extracts of lamb meat were repeatedly negative. However, when lamb meat was incubated with the patient's gastric and duodenal juice in succession, the resultant substance gave a positive reaction on skin test and on passive transfer, whereas tests with gastric and duodenal juice alone were negative.

However, there is also some evidence that failure to demonstrate positive skin tests in certain cases of clear cut clinical food allergy may not be due to changes in the antigens as a result of digestion but merely failure to employ, for testing, the antigens in an active form. Tuft and Blumstein[7] have demonstrated that the antigens of berries and certain other fruits were easily destroyed in the routine preparation of extracts for skin testing. In patients with negative skin tests to standard extracts, but with known clinical sensitivities to these foods, scratch tests with the fresh juice evoked positive skin tests.

More recently Ancona and Schumacher[8] have demonstrated a similar phenomenon in patients allergic to shellfish. In 14 patients definitely clinically allergic to shrimp, three different commercial extracts gave little or no reaction on skin test, but fresh or quick-frozen material gave positive reactions on scratch tests. They, therefore, concluded that fresh or quick-frozen materials are superior to standard extracts for skin testing. In a later report, Ancona and his associates[9] demonstrated positive scratch tests to crab meat in a case of purpura although tests with the corresponding commercial extracts by scratch and intradermal tests were negative. According to the authors, this may possibly be due to the presence of a themolabile factor in fresh food which either is removed by Seitz filtration and, therefore, absent in commercial extracts, or deteriorates in time at room temperature. It will be of interest to see this problem developed further with a greater variety of foods.

From the foregoing it is evident that there is a difference of opinion among investigators regarding the explanation for negative results of skin tests with standard extracts of foods which produce definite clinical reactions after ingestion. Since the majority of such clinical reactions associated with negative skin tests are of a delayed type, this investigation is concerned with the effect of digestion of foods on the antigenicity of such foods, as indicated by the skin

test, by the correlation of such skin test reactions with the results of intentional feeding trials, and the rapidity of such clinical reactions as occurred. We have attempted to determine whether or not digestion altered the antigenicity of a food so that the resulting complex was capable of producing a positive reaction on skin testing, which was not elicited by the unaltered food. If this occurred, the clinical reaction following the ingestion of the food should be of the delayed type, the delay theoretically being due to the lapse of time required for the production by digestion of the antigen to which the individual was sensitive and which was the actual cause of the clinical reaction.

MATERIALS USED

The following ten foods were utilized for the study: cocoa, orange, beef, wheat, milk, egg, chicken, corn, potato, and string bean. Extracts of these foods were made in the usual manner and standardized according to the protein nitrogen unit system. Digests of each of these foods, similarly standardized, were prepared* as follows:

Three hundred grams of food were placed in one liter of water and sodium hydroxide was added to pH 7.5. The extraction was continued for two hours. At this time the mixture was acidified to pH 2.5 with hydrochloric acid, 0.1 Gm. of pepsin was added and the mixture was incubated at 50° C. for 24 hours. A 300 ml. aliquot was then removed, the pH adjusted to 5, and the material boiled and filtered. The pH was then adjusted to 7 and Merthiolate was added in a 1-10,000 concentration. This was labeled the pepsin digest.

The remainder of the initial extraction was then adjusted to pH 8.5 and 10 ml. of chloroform and 1 Gm. of trypsin were added to it. Digestion proceeded for 24 hours at 40° C. The mixture was then boiled, centrifuged, filtered, and the pH adjusted to 8. Merthiolate was added in a 1-10,000 concentration, and this was designated the trypsin digest.

PROCEDURE

Thirty patients with various types of allergic disorders were picked at random for testing with the ten foods under study. Each patient was tested with the standard extract, pepsin digest, and trypsin digest in equal concentrations. Tests were made by the intracutaneous method and were read after an interval of twenty minutes. The skin test reactions were recorded as slight, moderate, or marked.

After the completion of all tests, each patient was given an intentional feeding test with each of the foods under study. Where the clinical trial was positive, the time interval between the ingestion of the food and the onset of clinical symptoms was noted. Differences in skin test reactions between the digests and the standard extracts were noted and correlated with the clinical reaction time. Of special importance in this study were positive skin reactions to one or both digests of a food of which the standard extract gave a negative skin test.

*Grateful acknowledgment is made to Mr. S. L. Shapiro, Director of the Biological Laboratory of the Arlington Chemical Co. of Yonkers, N. Y., for the preparation of the food digests used in this study.

FINDINGS

Of 125 negative tests with standard extracts, 56 were negative on tests with both the pepsin and trypsin digests, 26 were positive on test with the pepsin digest, 29 positive on test with the trypsin digest, and 14 were positive with both pepsin and trypsin digests. The majority of the positive reactions were slight reactions. Thus this group included many instances of negative skin reactions to standard extracts associated with positive reactions to the digests of the same foods.

Of the 195 positive reactions to skin tests with the standard extracts, 75 were positive to the standard extract alone, 34 were positive also with the pepsin digest, 41 to the trypsin digest, and 45 to both the pepsin and trypsin digests. Thus, 116 were negative to the pepsin digest, and 109 were negative to the trypsin digest. In this group, positive reactions to the artificial food digest extracts may be due to the incomplete digestion of the foods during the process of artificial digestion. These results are summarized in Table I.

TABLE I. RESULTS OF SKIN TESTS WITH STANDARD EXTRACTS OF 10 COMMON FOODS AND WITH THE PEPSIN AND TRYPSIN DIGESTS OF THESE FOODS IN 30 ALLERGY PATIENTS

Total Skin Tests Recorded	320	
Positive skin tests with standard extracts		195
Negative skin tests with standard extracts		125
Positive Skin Tests with Standard Extracts	195	
Positive also with pepsin digest extract		34
Positive also with trypsin digest extract		41
Positive also with trypsin and pepsin digest extracts		45
Positive with standard extract only		75
Negative Skin Tests with Standard Extracts	125	
With positive skin tests with pepsin digest extract		26
With positive skin tests with trypsin digest extract		29
With positive skin tests with pepsin and trypsin digest extracts		14
With negative skin tests with pepsin and trypsin digest extracts		56

Of 268 intentional feeding tests with the ten foods under study, 16 yielded positive clinical trials. Of the 16 positive clinical reactions, 11, or 4 per cent, occurred with foods giving positive skin reactions to the standard extracts, and 5, or 2 per cent, occurred with foods giving negative skin tests to the standard extracts. Of the 11 positive clinical trials with foods yielding positive skin reactions to standard extracts, 7 gave positive reactions on skin test to the digest extracts also, while in 4 cases, the skin test which was positive with the standard extract was negative with the digests. Of more importance, of the 5 positive clinical trials in which skin tests with the standard extracts were negative, the skin tests with the digest extracts were negative in 2 cases, but were positive in 3 cases. The results are summarized in Table II.

TABLE II. RESULTS OF 268 INTENTIONAL FEEDING TESTS IN 30 ALLERGY PATIENTS WITH POSITIVE OR NEGATIVE SKIN TEST REACTIONS TO STANDARD, PEPSIN DIGEST AND TRYPSIN DIGEST EXTRACTS OF 10 COMMON FOODS

Total Intentional Feeding Tests	268		
Negative feeding tests		252	
Positive feeding tests		16 (6%)	
Résumé of Positive Intentional Feeding Tests With Foods in Relation to Skin Tests:			
Number of positive feeding tests	16		
With positive skin tests to standard extracts		11 (4%)	
Together with positive tests with digest extracts			7 (2.6%)
Together with negative tests with digest extracts			4 (1.6%)
With negative skin tests to standard extracts		5 (1.9%)	
Together with positive tests with digest extracts			3 (1.1%)
Together with negative tests with digest extracts			2 (0.8%)

Of the 16 positive reactions to clinical trials, 8 were of the immediate type, with onset of symptoms in 5 to 30 minutes. In this study, clinical reactions, with the onset of symptoms within one hour, were considered immediate reactions, while those occurring after one hour, were considered delayed reactions. Of interest in this group is the fact that in 2 instances the skin tests were negative with the standard extracts, but positive to the digest extracts, whereas in 2 other instances the skin tests to all extracts, both natural and digests, were negative. The remaining 8 clinical trials were of the delayed reacting type. Of interest in this group is the fact that, in 5 cases, tests were positive to both the standard and digest extracts, and in two other instances there was a delayed clinical reaction in which positive skin tests were elicited with the standard extract only. The one remaining case in this group is the only one which conforms to all the criteria of the theory of specific allergy to products of digestion of foods, having a delayed clinical reaction, negative skin test with the standard extract and positive skin tests to the digests. These results are summarized in Tables III and IV.

TABLE III. RELATIONSHIP BETWEEN THE POSITIVE CLINICAL REACTIONS AND THE SKIN TESTS WITH STANDARD AND DIGEST EXTRACTS OF 10 FOODS

	IMMEDIATE REACTION	DELAYED REACTION
With positive skin tests to standard extracts only	2	2
With positive skin tests both to standard extracts and to digest extracts	2	5
With negative skin tests to standard extracts but with positive reactions to digest extract	2	1
With negative skin tests to both standard extracts and digest extracts	2	0
Number of Positive Clinical Reactions	16	

TABLE IV. POSITIVE REACTIONS TO FEEDING TESTS

PATIENT	DIAGNOSIS	FOOD TESTED	SKIN TEST RESULTS		TYPES OF CLINICAL REACTION	REACTION TIME	DURATION OF REACTION	SYMPTOMS OF REACTIONS
			STANDARD EXTRACT	DIGEST EXTRACT				
1. R.S.	Asthma	Cocoa	#	O	Immediate	10 min.	20 min.	Cough
2. N.G.	Asthma	Cocoa	O	#	Immediate	30 min.	30 min.	Itching of body
3. G.F.	Asthma Rhinitis	Cocoa	#	O	Immediate	5-10 min.	3-4 hrs.	Wheezing, nasal itching, and clogging
4. N.G.	Asthma	Orange	O	O	Immediate	30 min.	1 hour	Wheeze and cough
5. R.D.	Rhinitis	Milk	#	#	Immediate	30 min.	24 hours	Itching rash on neck rhinorrhea, cough
6. R.S.	Asthma	Wheat	O	#	Immediate	30 min.	5 min.	Cough
7. L.S.	Eczema	Orange	O	O	Immediate	10 min.	3-4 hrs.	Rash on face
8. E.H.	Asthma	Stringbean	##	#	Immediate	30 min.	15 min.	Rhinorrhea after fresh and canned stringbeans
9. J.S.	Asthma	Cocoa	#	O	Delayed	3 hours	2-3 hrs.	Heaviness on chest
10. L.K.	Hay Fever Eczema	Orange	O	#	Delayed	6 hours	1 hour	Intense itching of eyes
11. N.G.	Asthma	Milk	#	#	Delayed	3 hours	12 hours	Itching scalp with scab formation
12. F.R.	Allergic arthritis	Orange	#	#	Delayed	12 hours	All Day	Painful, swollen joints of hand
13. L.G.	Asthma	Corn	#	#	Delayed	2 hours	24 hours	Sneezing, rhinorrhea
14. M.A.	Neuro-dermatitis	Corn	##	#	Delayed	12 hours	3-4 hrs.	Body itch
15. S.B.	Asthma	Stringbean	##	#	Delayed	2 hours	12 hours	Heaviness on chest
16. J.S.	Asthma	Stringbean	#	O	Delayed	5 hours	8 hours	Heaviness on chest, asthmatic attack relieved with ephedrine

0 = Negative Reaction; # = Slight Reaction; ## = Moderate Reaction.

DISCUSSION

It is conceded at the outset that the number of foods used in this study was limited and that the number of patients tested was small. Since, however, this study was concerned with the problem that digestion could alter the chemical composition of a food in such a way that the end result may prove to be the provocative agent in cases of food allergy, we sought confirmation of this from the effect of such food digestion on the skin test. The opinion has been expressed by others that certain delayed clinical reactions to foods are due to the effects of digestion upon foods and that the patient is not sensitive to the unaltered protein of the food eaten, but to the product of its digestion. We attempted to demonstrate sensitivity to digests of foods by means of the skin test and to confirm such sensitivity with the intentional feeding test, necessarily of the delayed type.

Changes in food skin test reactions from negative to positive, as a result of food digestion, suggest that a new antigen has been formed which is responsible for the clinical response. There were numerous instances in this study of changes in skin reactions with the digest extracts, but these were not confirmed clinically by intentional feeding tests. Of a total of 268 clinical trials, 16, or 6 per cent, were positive, compared to 3.1 per cent in our previous study.[10] Of these 16 positive trials, 11, or 4 per cent, occurred with foods giving positive skin reactions, compared with 4.5 per cent in our previous study.[10] Out of the 16 positive trials, 5 occurred to foods that yielded negative skin tests with standard extracts, an incidence of 2 per cent as compared to 1.9 per cent in our previous study.[10] In 3 of these 5 cases, the positive clinical food trials occurred with foods that reacted negatively in the skin with the standard extracts but gave positive reactions with the digest extracts. However, in only one of these 3 positive clinical trials did the clinical reaction appear as a delayed reaction. In the other 2 trials the reactions were of the immediate type. This emphasizes the infrequency of that type of food allergy resulting from sensitivity to products of food digestion.

However, some of the findings in this study are confusing and difficult of explanation. In two instances there occurred positive clinical reactions of the immediate type to foods giving negative skin tests to both standard and digest extracts. The mechanism of this type of reaction is unknown, and the explanation commonly offered is that the shock organ is sensitive and reactive on contact with the allergen, while the skin as an organ has not as yet become sensitive to provide the positive skin test. Tuft and Blumstein,[7] as well as Ancona and associates,[8, 9] regard these as a type of false negative reaction due either to the loss of the antigenic fraction during the routine preparation of extracts, or to the presence of a thermolabile fraction in the antigen which is destroyed on exposure to room temperature. On the other hand, there were 5 instances of positive clinical reactions of the delayed type to foods which gave positive skin reactions with both the standard and digest extracts. It is possible that the delayed reactions were due to the cumulative effect of the gradual absorption of

the unaltered food as well as the products of the digestion, since all extracts reacted positively in the skin and the individuals were presumably sensitive to a slight degree to the unaltered foods and their digestion products.

Two delayed reactions with foods yielding positive skin tests with the standard extracts only, may have been, in reality, immediate type of reactions, occurring three hours after ingestion of the foods. There is some confusion as to what constitutes a delayed reaction. Cooke[11] states that the immediate reaction is one that occurs within one hour after contact with an antigen, but in describing a delayed reaction[11] he states that the delayed reactions are those occurring several hours to several days after contact with the allergen. Again he states[11] that the skin sensitive group is characterized by a swift reaction time varying from a fraction of a second to several hours between contact with the exciting substance and the development of symptoms, while the food sensitive cases negative by skin test do not develop symptoms until an interval usually of 24 hours or longer has elapsed following the ingestion of the offending food. In this study, clinical reactions from intentional feedings occurring within one hour have been interpreted as immediate reactions, while those occurring beyond one hour, as delayed reactions.

That clinical symptoms can and do occur from foods yielding negative skin reactions is admitted by most observers. In many such instances the reaction is of the delayed type, and if due to the result of food digestion, the delay should be equal to the period of time necessary for the food digestion and the production of a new antigen. The incidence of such delayed clinical reactions, due to food digestion, is not stated by other observers but from the results of this study they are very few in number. From the results of this study, it is evident that the food digestion theory is not the explanation for the many cases of asthma, urticaria, rhinitis, or migraine with negative skin tests. Cases that can be proved to be due to specific sensitization to the products of food digestion appear to be limited in number.

The results of this study with 268 clinical food trials, when compared with our previous study[10] with 2,467 clinical food trials are so similar as to confirm our previous conclusions that foods play a minor role to pollens, inhalants and infection in the majority of allergic individuals.

SUMMARY

Thirty patients with various allergic manifestations were tested with standard extracts and with the pepsin and trypsin digest extracts of ten common foods.

Confirmatory clinical trials with the ten foods were made for each skin test, both positive and negative, by means of intentional feedings.

The results of the skin tests with the standard extracts, compared with their pepsin and trypsin digests, are suggestive of changes in the antigenicity of foods due to digestion.

Out of 16 positive feeding tests, however, clinical confirmation of a change in the antigenicity of foods due to digestion was obtained in only one instance

wherein the standard extract failed to react in the skin, the digest extracts reacted positively, and the positive clinical reaction with the intentional feeding was of a delayed type.

The results of this limited study suggest that changes in the antigenicity of foods as a result of digestion, as indicated by skin tests and intentional feeding tests, are an infrequent occurrence. In the search for the etiologic agent in nonreactive cases, the use of digest extracts for testing purposes will offer no advantage over a thorough and carefully taken history, confirmed by test feedings.

This study confirms our previous conclusions that foods play a minor role to pollens, inhalants, and infection in cases of allergy.

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MERCUHYDRIN SENSITIVITY

Report of a Case

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MERCURIAL diuretics have become indispensable therapeutic agents in the management of congestive heart failure. Fortunately, the incidence of undesirable reactions resulting from their use is low, and the majority have taken place after intravenous injection.[1]

Recently we have observed a systemic allergic reaction to the intramuscular administration of Mercuhydrin. This compound,[2] which became available for clinical use in 1945, is the sodium salt of meralluride (mercurated allylsuccinylurea) and theophylline. Each cubic centimeter contains the equivalent of 39 mg. of mercury in organic combination and 48 mg. of theophylline (U.S.P.). Its chief advantage has been the absence of irritation upon intramuscular injection, although administration by this route has not prevented entirely the occurrence of severe reactions. Even so, the relative safety of this drug is attested by the paucity of unfavorable clinical reports in seven years of extensive use.

CASE REPORT

P. S. M., a 68-year-old male school principal, was admitted to St. Luke's Hospital on Dec. 18, 1949, in congestive cardiac failure due to arteriosclerotic and hypertensive heart disease. There was a history of generalized urticaria in 1944 following the use of sulfanilamide, but this episode had been the only manifestation of allergic disease.

The patient was an extremely large man of more than six feet and well over two hundred pounds. The temperature was 98.2° F., the pulse 68, and the blood pressure 180/66. He was dyspneic on the slightest exertion and when flat in bed. Examination disclosed pallor of skin and mucous membranes, distended neck veins, cardiac enlargement, forceful heart sounds, a soft systolic murmur at the apex and over the base, regular rhythm with occasional extra-systoles, signs of considerable fluid in the right pleural space, an enlarged liver extending well below the costal margin, marked edema of the abdominal wall, sacral region, and thighs, and massive swelling of the lower legs and feet. The rectum contained large internal hemorrhoids, one of which was thrombosed. Proctoscopy revealed no abnormality as far as the sigmoid flexure. Hematologic study revealed a moderately severe hypochromic anemia which had apparently been produced by chronic blood loss from the hemorrhoids. Immediate supportive measures and hemorrhoidectomy later were sufficient to correct this abnormal state. Other laboratory data included a normal leukocyte count and differential, a normal urinalysis, and stools positive for occult blood. Blood chemical determinations gave values of 34 mg. per 100 ml. for nonprotein nitrogen, 5.6 Gm. per 100 ml. for total protein (albumin, 3.4, globulin, 2.2), and 7 units for icteric index. Electrocardiographic interpretation indicated nodal rhythm, myocardial degeneration, and digitalis effect. Chest roentgenogram confirmed the impression of a large right hydrothorax and generalized increase in size of the cardiac shadow. Barium study of the entire gastrointestinal tract failed to demonstrate any abnormality.

The usual measures for treatment of heart failure were instituted and a maintenance dose of digitalis was continued. The admission weight was 245 pounds. Two milliliters of Mercuhydrin were given intramuscularly on the first and second hospital days; 1 ml. on the third and

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Received for publication June 9, 1952.
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fourth. There was excellent diuretic response, twenty-seven pounds being lost during a five-day period. One liter of straw-colored fluid was aspirated from the right pleural space the day after the patient was hospitalized.

A fifth dose of Mercuhydrin (1 ml.) was administered intramuscularly at 10:00 A.M. on December 23, the sixth day after the initial injection. Ten hours later at 8 P.M. there was a shaking chill. The patient became intensely cyanotic, extremely dyspneic (lungs were clear except for residual fluid in the right pleural cavity), apprehensive, confused, and incontinent of urine. Morphine and oxygen were administered immediately. The acute symptoms subsided within an hour. However, the temperature had risen to 102.8° F. at 10:00 P.M. By 4:00 P.M. of the next day the temperature had receded to 99.6° F. and continued at about this level for twenty-four hours, being entirely normal thereafter. The nonprotein nitrogen was 40 mg. per 100 ml. on December 24 and the patient appeared to have completely recovered. The significance of this episode was not accurately interpreted.

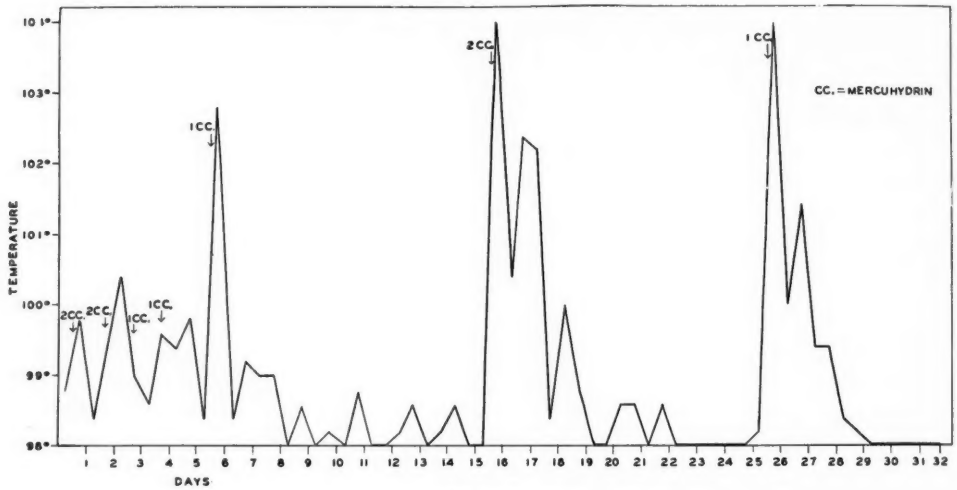

Fig. 1.

The course was uneventful and no fever was recorded until January 3, the sixteenth hospital day. On this date the temperature at 8:00 A.M. was 98° F. Two milliliters of Mercuhydrin were injected intramuscularly at 10:30 A.M. Approximately three hours later, at 1:40 P.M., the patient was suddenly seized with a severe, hacking, nonproductive cough. There followed in rapid succession a shaking chill, intense cyanosis, shortness of breath (the lungs remained clear), and mental confusion. The blood pressure was 150/0. Oxygen and morphine relieved the symptoms promptly. The temperature rose progressively to a peak of 104° F. by 8:00 P.M. and remained at this level for five hours, gradually receding to 100° F. by the next morning. Another rise occurred on January 4 to 102.4° F., but by 4:00 P.M. of the following day the temperature had returned to a normal level. Moderately severe hiccoughs developed on January 4. There appeared to be complete recovery by the afternoon of January 5. Again the significance of this reaction was not fully understood.

Subsequently, the patient was afebrile and without symptoms. Because of a gradual weight increase to 225½ pounds, 1 ml. of Mercuhydrin was again injected intramuscularly at 11:30 A.M. on January 13, the twenty-sixth hospital day. He complained of chilliness two hours later at 1:35 P.M. When examined at 2:30 P.M., he had vomited copiously, was undergoing a shaking chill, and appeared exceedingly apprehensive. There was intense cyanosis and marked dyspnea. He gave the appearance of one who was about to expire. The blood pressure was recorded as 160/40. The pulse was 96 and grossly irregular. The respiratory rate was 32. Oxygen was started and morphine was given immediately. Within an hour the

patient was considerably improved, cyanosis and apprehension having subsided, and the respiration was less rapid. The lungs were clear at all times. The temperature rose to 102° F. at 4:00 P.M. and reached a peak of 104° F. at 5:00 P.M. The fever then subsided gradually, being 100° F. at 7:00 A.M. on January 14. There was a second rise to 101.4° F. at noon on this date but a return to normal by 8:00 P.M. Again there was complete recovery.

We were now convinced that further administration of Mercuhydrin would be most hazardous. Consequently, no reaction occurred during the remainder of his stay in the hospital. The patient was discharged on Feb. 2, 1950.

During the ensuing year the patient remained well compensated and ambulatory on salt restriction, digitalis, xanthines, and ammonium chloride. These measures have not been sufficient, however, in recent months. When it became evident that mercurial diuresis would be necessary again, Salyrgan-Theophylline was given intramuscularly in gradually increasing dosage from 0.1 ml. to 1 ml., and at the time of writing this drug was being administered in a dosage of 1 ml. every two to four weeks. There has been no reaction and the diuretic effect has been excellent. It will be noted that Gelfand's[3] patient also tolerated Salyrgan intramuscularly without evidence of hypersensitivity after similar violent reactions to Mercuhydrin.

The striking febrile reaction, the associated cardiorespiratory and cerebral symptoms which followed by several hours the intramuscular injection of 1 ml. of Mercuhydrin on the sixth day after the first dose of this drug, and the occurrence of identical febrile reactions after each subsequent injection indicate that our patient had become sensitized to Mercuhydrin.

The patch-abrasion test[4] was utilized in an attempt to establish more specifically an allergic basis for the clinical manifestations observed. This procedure produced no local or systemic effects to full strength ampule solution of Mercuhydrin. Since it was not carried out until twelve months after hospital discharge the failure to elicit a positive response may possibly have been due to delay in testing.

Approximately twenty-seven months had elapsed since the third and last violent febrile paroxysm when, in accordance with the Leftwich technique[10] as successfully used by Gelfand,[3] we obtained blood serum from a patient receiving Mercuhydrin intramuscularly for the treatment of chronic heart failure. The intradermal injection of 0.05 ml. of this serum into the skin of the right forearm of our patient produced within fifteen minutes an area of edema with surrounding erythema and even small pseudopodia, which persisted for approximately twenty minutes before beginning to fade. Normal serum injected simultaneously in the opposite forearm as a control caused only a transient erythema which disappeared rapidly. From the result of this investigation it would seem logical to conclude that our patient remained allergic to Mercuhydrin for more than two years after the sensitizing dose.

COMMENT

Reactions resulting from acquired sensitivity to Mercuhydrin have been reported in eleven patients. Cohn[8] observed four; Wallner and Herman,[7] two; Modell, Gold, and Clarke[5]; Gelfand,[3] Fineman and Rosenberg,[6] Shatan,[1] and Reeves,[9] one each. There was one fatality.[7]

Typical of this group is the patient observed by Gelfand[3] who developed apprehension, difficulty in breathing, palpitation, choking, chest oppression, chills, fever, and sweating after Mercuhydrin intramuscularly, but was able to tolerate Salyrgan without reaction. Reducing the dose of the drug did not alter the patient's response, thus indicating, Gelfand believes, a qualitative allergic effect rather than a quantitative toxic one. As in our patient, Mercuhydrin had been administered a number of times before any untoward signs appeared. Direct skin tests with undiluted ampule solution of Mercuhydrin and Salyrgan gave negative results. The Leftwich technique,[10] in which blood serum obtained from another patient previously treated with Mercuhydrin was used for testing, provoked a positive skin reaction with marked erythema and pseudopodia.

From a review of these eleven cases certain outstanding features can be listed:

1. Febrile paroxysms usually accompanied by chills occurred repeatedly in seven patients.[1, 3, 5, 6, 7, 9]

2. Cutaneous phenomena appeared in a few instances.[6, 7, 8] The Negro woman reported by Fineman and Rosenberg,[6] in addition to febrile reactions following the eleventh, thirteenth, fourteenth, fifteenth, and sixteenth injections, developed an exfoliative dermatitis which lasted two to three weeks. Wallner and Herman's[7] patient had a transient cutaneous erythema on two occasions before the fatal dose. In one of Cohn's[8] cases there appeared a diffuse erythematous rash which progressed to exfoliation.

3. Reactions do not follow the initial injection but occur at variable intervals, usually six to seven days, thereafter. Often during this period there have been several well-tolerated doses. Gelfand[3] is of the opinion that "the preliminary administration of the drug sensitizes the patient, thus setting the stage for the outburst of the allergic response."

4. The severity of toxic response is independent of the amount given.

5. Mild subjective symptoms, slight temperature rises, and transient cutaneous eruptions must be considered as danger signals, sufficient to warrant discontinuance of the drug. Acute specific febrile reactions obviously contraindicate its further use.

6. Hypersensitivity to Mercuhydrin may not necessarily extend to other mercurial diuretic drugs. Salyrgan,[3] Mercupurin,[5] and Thiomerin[9] have been administered successfully to patients who previously reacted violently to Mercuhydrin.

7. Skin testing in patients who have developed sensitivity is rarely helpful.

8. A desensitization program may be successful as Shatan's[1] case has demonstrated. After being desensitized this patient tolerated Mercuhydrin without ill effect once or twice weekly until his death from heart failure six months later.

Mercuhydrin is an excellent product and one of several which play an exceedingly important role in the treatment of heart failure. We do not wish to convey the impression that its exhibition in clinical practice should be curtailed. However, it behooves us to be aware of the potential allergenicity of this drug when administered by the intramuscular route and to familiarize ourselves with the undesirable effects which occasionally may be severe and serious.

SUMMARY

1. A patient who experienced violent delayed febrile reactions after the fifth, sixth, and seventh intramuscular injections of Mercuhydrin has been reported.

2. The literature dealing with the manifestations of acquired hypersensitivity to intramuscularly administered Mercuhydrin has been reviewed.

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1000 W. GRACE ST.

AN INTRODUCTION TO THE PHYSICAL TECHNIQUES OF ELECTROPHORESIS AND ULTRACENTRIFUGATION*

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THE techniques of electrophoresis and ultracentrifugation which I will attempt to describe depend on very nonspecific properties of a particle. The usefulness of electrophoresis depends on the fact that many of the molecules that interest us such as proteins and polysaccharides carry electric charges. In ultracentrifugation we take advantage of the fact that all particles have mass and will be affected by centrifugal force. Both of these techniques, because they depend for their operation upon these very general and nonspecific characteristics of molecules in solution, have had and will continue to have wide application in chemistry and biology. What I shall attempt to do is tell a little of how these instruments work, the principles upon which their use is based, and a very little of the results that my colleagues and I have obtained in applying these techniques to problems of immunologic and allergic interest.

Let us first take up the technique of electrophoresis. As you all know, if one places a charged particle in solution between a positively charged electrode and a negatively charged electrode, or, in other words, if one places a charged particle in an electric field, the particle will move. If positively charged it will be repelled by the positive electrode and move toward the negative electrode. If negatively charged it will be repelled by the negative electrode or pole and be attracted to the positive pole. Electrophoresis, or cataphoresis as it is sometimes called, may be defined as the migration, in solution, of charged particles in an electric field. The speed with which the particle moves will depend on the voltage drop between the two electrodes and the amount of charge carried on the particle.

Studies in electrophoretic behavior date back as early as the first half of the nineteenth century, over one hundred years, but the real development of the field came from the pioneering work of Tiselius published in 1937. Since that time a large number of applications of the technique have appeared using the so-called "Tiselius apparatus." Moreover, many modifications have been used for special purposes: microelectrophoresis, paper electrophoresis, electrophoresis convection, and various types of preparative electrophoresis. We shall, however, confine our attention to the apparatus essentially as designed by Tiselius and improved by Longsworth and Philpot.

Fig. 1 shows the "business part" of the electrophoresis apparatus, the electrophoresis cell. This cell is only one of many types of cells. *E* is the bottom section, *D* the middle section, showing the two limbs or channels, *C* is the top section. The electrodes (*A*) shown here, when used, have silver wire

Presented at the Symposium on "Techniques of Experimental Immunology Applied to Allergy" in the Postgraduate Course of the American Academy of Allergy, Feb. 15, 1952. Other papers of the series will be published as space permits.

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Received for publication July 14, 1952.

*A part of the experimental work presented here was performed under a National Institute of Health Fellowship, 1949-1950.

wound around the bottom end and then going out the glass tube to the source of current. The wire is plated with silver chloride, making a silver, silver chloride electrode.

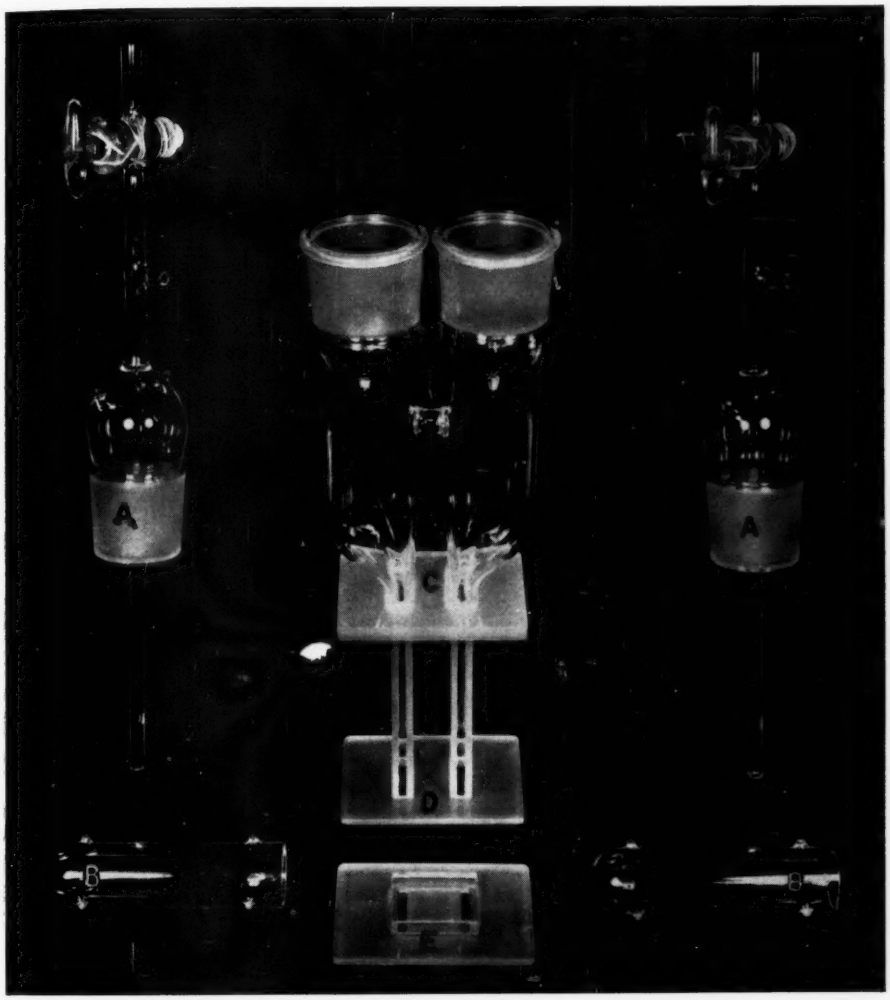

Fig. 1.—Electrophoresis cell and electrode. *A*, electrode; *B*, test tube; *C*, top section; *D*, middle section; *E*, bottom section.

The cell is placed in a metal rack in a water bath which is usually kept between 2 and 4° C. The rest of the apparatus consists of the optical parts designed so as to give us visible evidence of the migration of the charged particles in the cell under the influence of the current. There is a light source, the light being focused on a horizontal slit and sent through the window of the water bath, then through both limbs of the cell and then focused on a diagonal bar or diagonal slit, then through a cylindrical lens and then into a camera. I shall not attempt to explain the optics used but merely attempt to describe the basis for the results obtained with it.

Now let us start an electrophoresis experiment. We shall take a solution of protein, although any charged substance in solution will do, placing the protein in a cellophane sac and dialyzing the protein against a buffer solution containing the concentration of salt and hydrogen ion at which we have decided to run the experiment. Under certain circumstances this preliminary dialysis may not always be necessary although it frequently makes the interpretation of results simpler. The solution inside the sac is dialyzed until it has practically the same concentration of salt and pH as the buffer outside. We grease the end plates of the top, bottom, and middle sections of the cell and put them together so they do not leak and there is free communication between the channels. Placing the cell in the cell rack (not pictured) we take a syringe with a long needle, and add the protein solution until it is just above the bottom section and then slide over the bottom section so that it is isolated from the middle and top sections. From one limb the protein remaining in the middle section is removed, rinsed with buffer, and then buffer added all the way up into the top section. We add protein to fill the other channel and then move the top section so that the final result is that the channels in the bottom section are isolated from the channels in the middle section which in turn are isolated from the top section; and protein fills one channel and buffer the other. We then add buffer all the way up in the top section, put in the two test tubes (*B* in Fig. 1), and seat our electrodes in the test tubes. Through the electrodes we add concentrated salt solution, place the rack with the cell into the water bath and allow our solutions to come to the temperature of the bath. The electrodes are attached to a current source, the light is turned on, and after temperature equilibrium is reached the boundaries are formed. This last consists of moving, through a mechanism on the rack, the top and bottom plates back to their original place so that there is again free communication between the two limbs of the cell. On both sides of the cell we have buffer layered on the top of protein solution with a sharp interface or boundary between them. Because of the mode of formation of these boundaries they are behind the end plates and so are not visible. In order for them to be seen, pressure is placed on one side pushing one boundary down into one channel and up into another. This process is known as "compensating the boundary in." Once we have our boundaries in view they are ready to turn on the current; the experiment can now begin.

In making an experiment we would like to know whether in our protein solution there is present one or more than one charged component, how fast the component or components move, and the concentration of the component or components. If there is more than one component, we would possibly like to be able to isolate one or more of the components in an electrophoretically pure state. In order to obtain even a little understanding of how these ends may be accomplished we must have some comprehension of what happens when we turn the current on. This is diagrammed in Fig. 2.

In Fig. 2 we see the diagram of the cell filled with protein and buffer and the boundary compensated in. The dots represent the protein in solution. The positive and the negative electrodes are placed as shown by the + and – signs. The protein we shall suppose is negatively charged. The graph on the right of

the diagram of the cell shows the concentration of protein plotted against distance in the cell. At the top of the cell there is only buffer, i.e., the protein concentration is zero. Then coming to the boundary between the protein and buffer, the concentration rises immediately to the concentration of the protein solution. The practically instantaneous manner in which the protein concentration changes at the boundary from zero to the original concentration of protein is indicated in the second graph by the straight line going completely off the graph. Here is plotted the rate at which the concentration changes with distance and of course at the boundary the rate at which the concentration changes is infinite. The buffer and protein in the left-hand limb show the same.

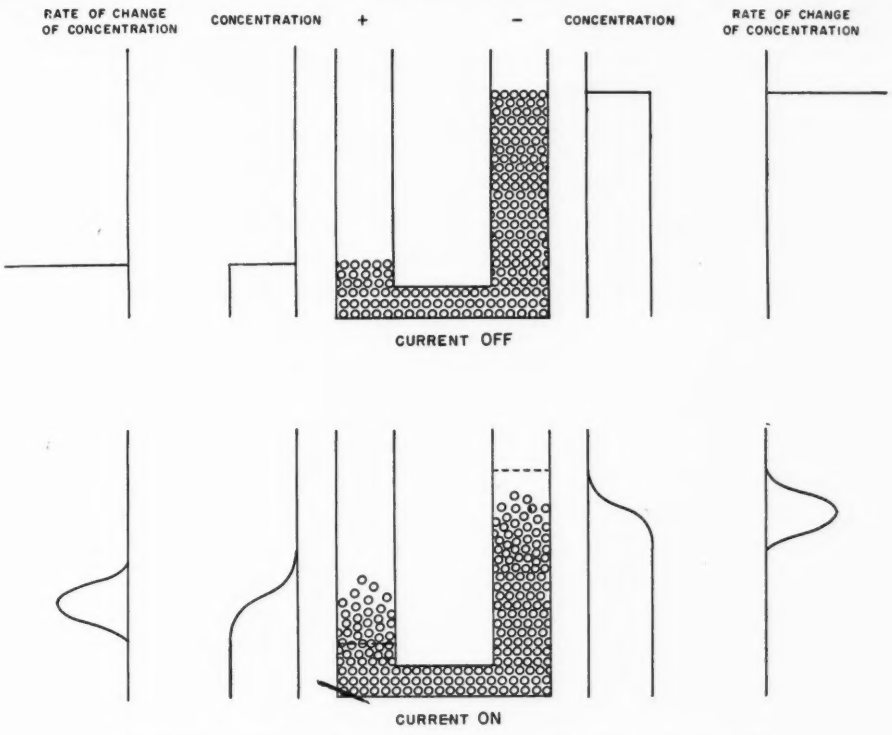

Fig. 2.—Electrophoresis with single negatively charged component.

Now let us turn on the current and wait an hour or so and see what has happened. This is diagrammed in the lower drawings. We can see that the protein being negatively charged moved away from the negative electrode down the cell, which is why this side is called the descending side. In the other limb, the ascending side, the negatively charged protein moves up toward the positive electrode. Notice that the original sharp boundary is somewhat diffuse or "blurred"; this is due partly to the diffusion of protein, which is independent of the electrophoretic process and possibly is partly due to the fact that some of the protein molecules might carry slightly less or slightly greater charge than other protein molecules, i.e., the protein is not quite electrophoretically homogeneous. This blurring of the boundary can be represented in the first right-

hand graph where we plot again the concentration of protein against distance in the cell. We see above, the region where there is only buffer and the protein concentration is zero; then a small amount of protein moving slightly less rapidly than the bulk of protein; then the protein concentration slowly but regularly increasing until we come to a region where the original concentration of protein is present. Notice that at low concentration of protein the curve of concentration versus distance changes very slowly and then changes more rapidly and then starts to slope gradually until the protein concentration becomes equal to the original and constant, i.e., the rate of change is zero. The differences in the rate at which the concentration of protein is changing are best expressed in the graph of rate of change of protein—concentration versus distance. Here we see

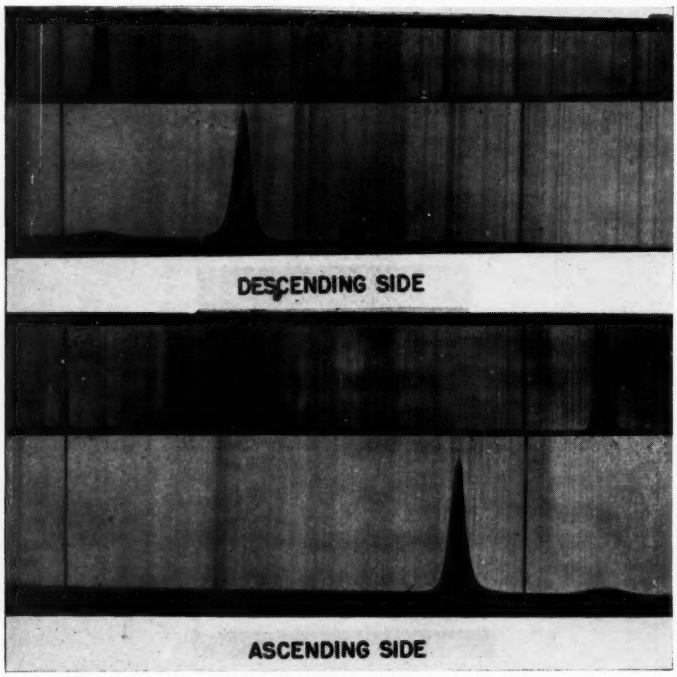

Fig. 3.—Electrophoresis—single component, human serum albumin.

that the rate at which the protein concentration changes first is slow, then faster, reaches a maximum, and again changes more slowly until finally here it no longer changes, i.e., its rate of change is again zero.

Now why do we bother with this troublesome concept of rate of change of protein concentration? Simply because the optical system which is used in the apparatus is designed to give, *under ideal conditions*, a direct picture of what corresponds to the rate of change of protein concentration versus distance in the electrophoresis cell.

This is seen in Fig. 3 which is the actual pictures obtained in the course of an electrophoretic experiment made on crystalline serum albumin. First is our starting picture of the albumin boundary with the line going off to infinity.

Then the current was turned on, and we see the peak of albumin as it has moved away from the starting boundary. The similarity between this peak of an electrophoretic component and the third diagram we drew in Fig. 2 is evident. One can see the pictures obtained on the ascending and descending sides. Notice the small peaks where the initial boundaries were. These small peaks are called the "stationary" or "false" boundaries because they do not move or move very slowly. They do not correspond to any true component in the solution. They are present in most electrophoretic patterns and can, under many circumstances, be ignored.

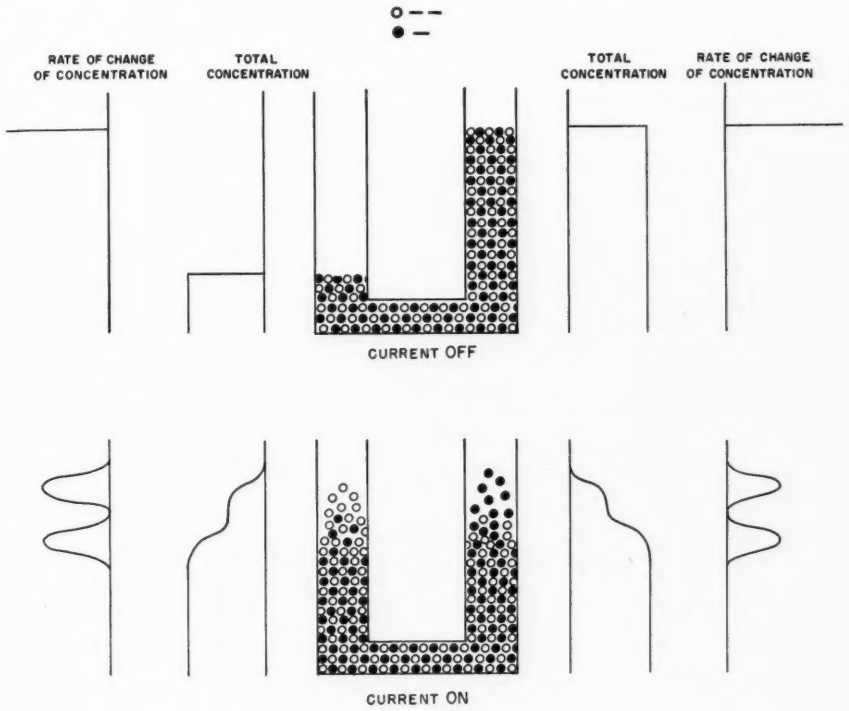

Fig. 4.—Electrophoresis with two negatively charged components.

Now, in Fig. 4, let us see what happens when we make an electrophoretic experiment with two components and for simplicity we will assume them both to be negatively charged. One of the components is represented by the open circle and carries a greater charge than the other represented by the solid dots. Before we turn on the current the situation is as in Fig. 2. Let us focus our attention on the descending side. When we turn on the current the open circles carrying more charge move into the bulk of solution leaving behind the slower solid dots until there is a portion of the cell where there are only solid dots. It is apparent if we take a syringe with a long needle and place the needle in this portion and draw solution off with the syringe and needle, we will get pure solid dots. Thus we can achieve one of our purposes: to isolate one of the components in an electrophoretically pure state. It is also apparent we could isolate the faster component in the same way on the ascending side.

Now let us see what has happened to the concentration of protein. Before the current was turned on the total concentration was as given in the diagram to the right, and the rate at which the concentration changes is assumed as before to be infinite. After the current has been turned on and resolution of the two components has occurred, we get the situation seen in the lower graph on the right. There is first the region of only buffer where the concentration of protein is zero and then proceeding farther down the cell we get into the region of closed circles and the concentration of this protein slowly starts to rise until finally a region is reached where the concentration of closed circled protein is constant and the same as it was in the original solution before electrophoresis. As we proceed still farther we come to a region where we begin to meet a few molecules of the faster-moving open circle protein and the concentration of this protein starts to rise slowly until we reach a point where it becomes constant and equal to the concentration of protein with which we origianlly began.

When we turn our attention from the manner in which the protein concentration varies with distance in the cell to the way in which the *rate* at which this concentration changes, we see the situation diagrammed in the second graph. We see two bell-shaped curves or peaks corresponding to the two components which are present. These two peaks are obtained in the same manner that the single peak was obtained in the preceding figure.

Not only do these peaks give us the position of the boundaries in the cell, but also under *ideal conditions* the areas under these peaks are proportional to the concentrations of the components represented by the peaks. If one knows the optical characteristics of the electrophoresis apparatus and certain characteristics of the components, one can obtain from the areas the respective concentrations in absolute terms, e.g., grams of protein per 100 ml. of solution. Very frequently this is not done but rather one finds the area under each peak. The areas under the individual peaks are proportional to the concentrations of the individual components and the sum of the areas is proportional to the total concentration. Therefore the fraction formed by dividing the area of a given peak by the total area under all peaks is proportional to the fraction which that given component makes up of the total concentration, i.e., the percentage concentration of that component. If one knows the total concentration in Gm. protein/100 ml. of solution, one can therefore find, in many cases, the concentration of the individual components in Gm. protein/100 ml.

The two-component situation is more or less exemplified in Fig. 5 where we see an electrophoretic pattern of a solution containing two types of components. In this experiment rabbit antibody to crystalline bovine serum albumin was specifically precipitated with bovine serum albumin and the precipitate washed with buffer until it was free from all nonspecific protein. Then the precipitate was dissolved in excess antigen. This solution of antigen and antigen-antibody complex was run in the electrophoresis apparatus in the manner that has been described. The leading peak is the free antigen, the trailing peak corresponding to antigen-antibody complex. Notice that the peak corresponding to antigen-antibody complex is not symmetrical, showing that there is not one complex

but probably several with rather similar rates of migration. The two upper patterns were obtained using a "bar" or knife edge in the optical path of the light. The lower pattern was obtained using a slit.

These patterns, however, bring up a question. How can I so confidently state that the leading peak corresponds to antigen? How are these various peaks identified? One of the ways is by obtaining the "mobility" of the several components. The speed of migration of a given component under fixed conditions is a stable characteristic of a given molecule and can be used to identify the molecule. Since, as was pointed out in the beginning, the speed of migration depends on the strength of the electric field, we always speak of the speed of migration of a particle per unit field strength. This is known as the mobility, the cm./sec. the boundary moves at a constant field strength of one volt/cm.

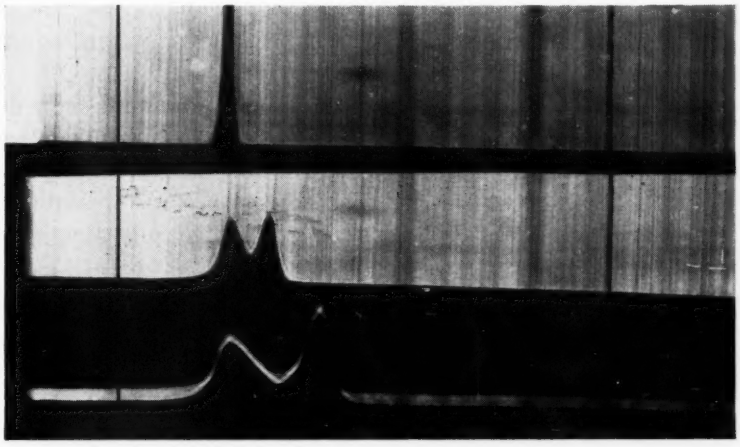

Fig. 5.—Electrophoresis experiments, two types of electrophoretic components.

This mobility not only depends on the particle in question, but also on the type and the concentration of buffer ions and on the pH of the solution. Therefore, we can characterize a charged substance electrophoretically by stating its mobility in a buffer containing given ions of a given concentration and at a given pH.

One can obtain quite a bit of information by making an experiment at a single pH. Frequently much more information can be gained by running a number of experiments at various pH's and if possible several ionic strengths. An example of what I mean are some experiments done on short ragweed. Short ragweed was extracted with water, dialyzed against a buffer of a given pH, and then run in the Tiselius apparatus. The mobilities of the various components were calculated. Then another extract was made, dialyzed against a buffer of a different pH, and run. This was repeated until a range of pH's was covered from pH 8.6 to pH 3.0. The results are seen in Fig. 6, where the mobility of each component is plotted against pH. Five charged components were present with more or less regularity. Notice the middle curve. At pH 8.0 it is negatively charged, but as we decrease the pH, i.e., increase the H+ ions in the environment of this molecule, more and more of the H+ ions combine with the

particle, lessening the negative charge on the molecule. As the net negative charge decreases the negative mobility decreases. Finally the H+ ion concentration is sufficiently great and sufficient H+ ions are pushed on the molecule so as to neutralize completely the negative charges; the molecule has no net charge. At pH 5.0 with no net charge, the molecule has zero mobility. This pH, where there is no net charge and zero mobility, is known as the isoelectric pH or isoelectric point. It is also a value characteristic for a given molecule. When we go to pH's lower than the isoelectric point even more + charges are pushed on the molecule, and the molecule assumes a positive mobility.

In Fig. 6 it can be seen that one component has an isoelectric point somewhere around 5 and another somewhere around 7. A third component is so highly basic, i.e., has so high an isoelectric point that even at pH 8.0 it is positively charged. The other two components are so acidic, i.e., have so low an isoelectric point that even at pH 3.0 they are negatively charged. Besides these mobile components there is, of course, the immobile "component" described by Abramson and others.

Because of the possibility of interaction occurring between these differently charged components during the electrophoresis run, it is possible that not all the peaks seen in the electrophoresis pattern of such a highly complex mixture as ragweed-pollen extracts represent true components. Correlated immunochemical studies make it probable, however, that the five components pictured in Fig. 6 are real.

The diagram of Fig. 6, however, gives one explanation of why there has been so much difficulty in fractionating ragweed. No matter at what pH one fractionates ragweed one is bound to have some components negatively charged and some positively charged so that combination between these oppositely charged components occurs and they tend to precipitate together. Moreover, from this diagram one can predict that attempts at fractionation of ragweed by such means as electrophoresis-convection will be largely fruitless.

Before closing our discussion of the electrophoresis apparatus I want to stress that, while I have repeatedly referred to proteins, electrophoretic techniques are not restricted to proteins or even large molecules. Electrophoresis has been applied to proteins, carbohydrates, nucleic acids, and even inorganic ions. The necessary property is that a particle carry an electric charge.

Now let us turn our attention very briefly to the use of the ultracentrifuge. All of you have used the ordinary laboratory centrifuge. One knows that if it is desired to sediment a large particle like a red cell one need only apply a relatively slow speed of centrifugation—1-2,000 r.p.m.—for a short time. For somewhat smaller particles such as bacteria a higher speed and longer time are required. Svedberg, a great Swedish scientist, was the first to build centrifuges capable of such speed that ordinary protein molecules could be sedimented in a reasonable length of time. This required centrifuges capable of speeds of 50,000 to 60,000 r.p.m. and exerting a centrifugal force of 100,000 to 200,000 times gravity or more. These centrifuges are called ultracentrifuges and have contributed very largely to our knowledge of large molecules in solution.

In order to obtain these high speeds the centrifuge rotors are driven by an oil turbine or air or (in one of the most popular commercial models) by an

electric drive. They have to be quite strong to stand the high forces generated and must have provision for running at known speeds and temperatures. They have in their rotor provision for a cell containing parallel transparent windows of glass or quartz into which a small volume, usually less than a cubic centimeter,

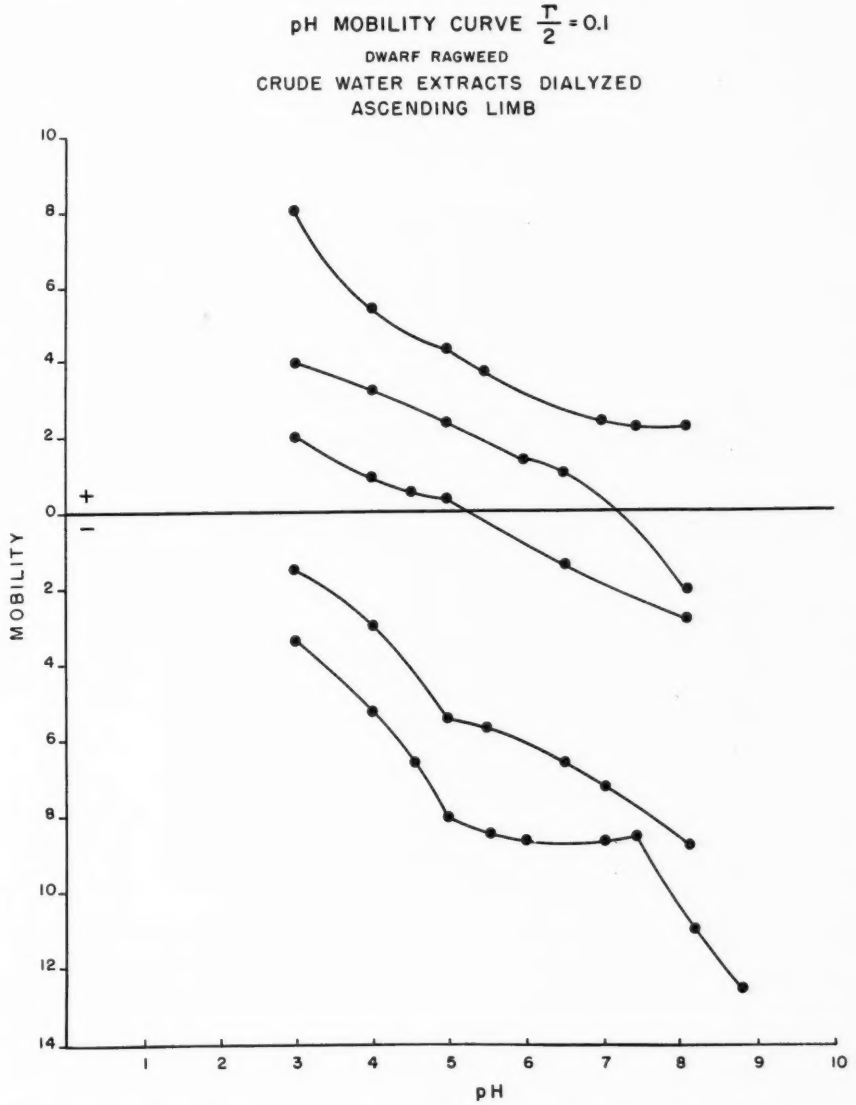

Fig. 6.—pH mobility curve $\mu = 0.1$. Dwarf ragweed, crude water extract dialyzed, ascending limb.

of the solution under examination is placed. These small volumes are used in the so-called analytical ultracentrifuges where we wish to examine a solution for the presence of components of different mass. Another type of ultracentrifuge is the preparative ultracentrifuge which can be used to fractionate a mixture into components which differ in their mass. We shall limit our discussion to

the analytical ultracentrifuge. In the ultracentrifuge components of different mass sediment with different speeds and set up boundaries of the same type which we saw in the electrophoresis apparatus.

Let us consider the situation where there is one ultracentrifugal component, i.e., there is only one component which has a sufficiently high molecular weight that it will sediment under the centrifugal force attained in the machine. As the machine gets up to speed the high molecular weight molecules tend to sediment away from the center of rotation, leaving behind the buffer ions and solvent molecules. A boundary is thus set up between the high molecular weight molecules and the solvent which is similar to the boundary we saw in electrophoresis.

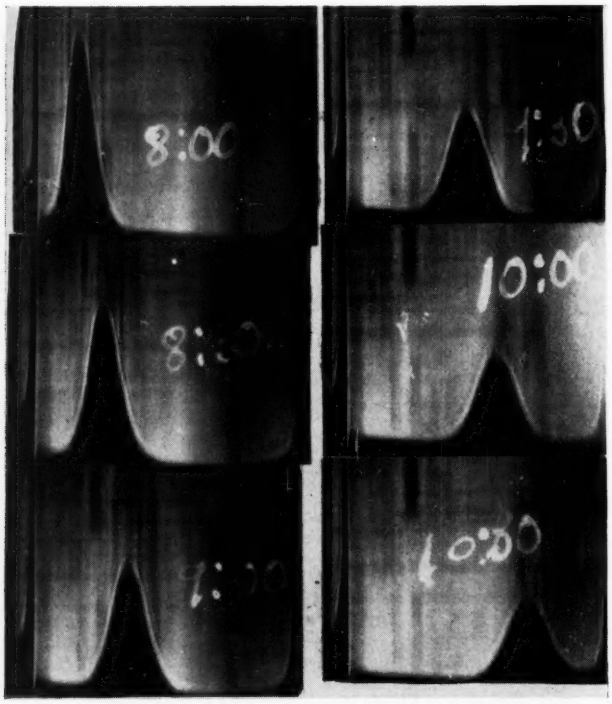

Fig. 7.—Ultracentrifuge experiment, single component, bovine serum albumin.

As these molecules move farther and farther into the cell, this boundary moves accordingly. The cell contains two transparent windows through which a bright light is shown. The cell is rotating so fast that persistence of vision permits an apparently continuous view of the cell contents. The same optical arrangement which is used to visualize the electrophoretic boundaries is used to visualize these sedimentation boundaries. The pictures obtained in an ultracentrifugal experiment in which one ultracentrifugal component is present are seen in Fig. 7. When two or more components are present which move at different rates more than one boundary is formed in the manner described for electrophoresis.

A given component is characterized in the ultracentrifuge by its sedimentation constant. This is the distance a particle moves/unit of time/unit angular velocity. The unit in which the sedimentation constants are expressed is the

Svedberg, corresponding to 10^{-13} sec. It has the symbol S. For a given particle the rate of sedimentation varies not only with centrifugal force but also with viscosity and temperature of the solvent, i.e., with the resistance the particle meets in its flow through the solvent. Therefore the sedimentation constants are corrected to 20° C. and the viscosity of distilled water. This is symbolized by s°_{20}.

In electrophoresis we can investigate whether the solution contains particles with the same or differing charges. The ultracentrifuge, on the other hand, can give us information as to whether a solution contains particles with the same or different mass or molecular weight. It is, of course, quite possible for components to have the same mass but different charge, or vice versa. Thus a given fraction may be homogeneous by ultracentrifuge but heterogeneous by electrophoresis, and vice versa.

Fig. 7 shows the ultracentrifugal pattern given by a preparation of crystalline bovine serum albumin as an example of a substance reasonably homogeneous in the ultracentrifuge. The black line on the left of each photograph is the meniscus of the solution and the peak corresponds to the bovine serum albumin boundary which in the topmost left-hand photograph has just broken away from the meniscus. The other photographs are taken at later and later times, and we see the boundary moving farther and farther into the cell. We measure the distance in centimeters moved by the peak in the interval of time from one picture to another. We divide the distance moved during this interval by the time in the seconds and divide this by the angular velocity and correct this to the viscosity of water at 20° C., and we have the sedimentation constant.

As in electrophoresis the area under the peaks, in the ideal case, is proportional to the concentration. By means of the ultracentrifuge, therefore, the number of components, their sedimentation constants, and their concentrations can frequently be obtained.

While it is generally true that the greater the mass or molecular weight the greater the sedimentation constant, one cannot obtain the molecular weight of a substance from the sedimentation constant alone. This is because for one reason the rate of sedimentation depends not only on the molecular weight but also on the density; e.g., fat globules or certain lipoproteins which are less dense than water will actually migrate upward. The rate of sedimentation depends also on the shape of the particle: a spherical particle goes through the solvent more smoothly than a nonspherical particle and will sediment faster than a nonspherical particle of the same mass. Similarly, the amount of water a particle carries with it affects its rate of sedimentation.

The literature on the ultracentrifuge and electrophoresis is vast but for those who wish to learn more the following will serve as an introduction.

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2. Amino Acids and Proteins, edited by David M. Greenberg, Springfield, Ill., 1951, Charles C Thomas, publisher, chap. 6 and 8.
3. Alberty, R. A.: An Introduction to Electrophoresis, J. Chem. Education 25: 426, 619, 1948.

Editorial

RESEARCH IN ALLERGY

MANY of the fundamental discoveries in the field of allergy have been the products of careful observation and active imagination, utilizing only the simplest techniques. The classic study of hay fever by Charles Harrison Blackley, establishing pollen as the causative agent, describing the scratch test and methods of making pollen counts, was the result of fourteen years' work of a busy practitioner using only the tools and materials familiar to all physicians of the time. His ability to advance medical knowledge depended on clear, logical thinking and careful experimentation rather than special technical methods. It is also worthy of note that the brilliance of Blackley's discoveries was in no way impaired by the fact that in the middle of his fourteen years of patient study, Henry Hyde Salter published his equally outstanding treatise on asthma, which covered some of the same material.

Fifty years later Prausnitz and Kustner demonstrated conclusively the immunologic or antibody mechanism of human allergy by an experiment more notable for its simplicity than for the scientific or technical knowledge required. By the method they established, many other workers, most of them practicing physicians, were able to make further important contributions to the understanding of allergic diseases. Even after thirty years, the possibilities of further advances by the use of passive transfer have not been exhausted; in fact, it remains the one standard method of demonstrating antibody activity in the sera of patients with allergic diseases of the hay fever-asthma group.

Meanwhile, advances in related fields of science have introduced new methods and broader concepts applicable to the study of allergic diseases. Immunology has made tremendous advances by the utilization of quantitative chemical methods and single purified antigens. The pathology of hypersensitive reactions has been greatly developed by the studies of Rice and others, while knowledge of their physiologic mechanisms has been expanded through studies of histamine and the antihistaminic drugs. In the case of asthma, understanding of the disturbance of respiratory function has been elaborated by quantitative measures of air flow, supplemented by cardiac catheterization and studies of blood gas concentrations. More recently the importance of endocrine factors, implied in the concepts of Hans Selye, has been concretely demonstrated in the clinical therapeutic use of cortisone and corticotropin.

All of these advances offer the promise of further experimental progress in the knowledge of the mechanism of human allergic diseases. However, they entail highly technical methods, whose full utilization requires specialized training and knowledge outside the scope of the average practicing physician, and in many instances complicated and expensive apparatus. Furthermore, the

application of quantitative immunochemical methods to the problems of clinical allergy is hampered by the fact that none of the clinically important allergens have proved to consist of a single pure antigen, so that before modern immunochemistry may be applied, a complex problem of protein separation must be undertaken. In the case of ragweed pollen, this problem has occupied several laboratories for a period of years.

It is apparent that fundamental research in the allergic diseases is becoming a problem for teams of highly trained workers in the basic medical sciences, rather than a diversion to which the busy practitioner can devote two or three afternoons a week. It will require the concentration in certain centers of able, imaginative brains, well grounded in science, and of the money to supply the needed equipment and supplies, under the direction of physicians who understand the broader problems of clinical allergy. It is to be hoped that the funds of the proposed foundation for allergic diseases will be wisely used to support fundamental research in institutions which have the scientific background and medical leadership to make basic contributions to the mechanisms of allergy. If they are to be used to support pollen and mold surveys or clinical studies of new drugs, the prospective contributors may rightly leave the burden to the hospitals, medical schools, and pharmaceutical manufacturers, who have already done well in these lines of investigation without outside help.

This does not mean careful study of allergic patients by ordinary clinical methods may not be rewarded by worth-while discoveries. However, most of the simpler observations have been made and chances of fundamental progress will be greater in those studies fully utilizing the most modern available methods.

American Academy of Allergy

Annual Meeting

The Ninth Annual Meeting of the American Academy of Allergy will be held at the Hotel Statler, Boston, Mass., Feb. 26 to 28, 1953. The scientific program is not yet available at the time of going to press but the committee reports an abundance of excellent papers on a wide variety of subjects covering both clinical allergy and related basic sciences. Dr. Chester S. Keefer, Wade Professor of Medicine at the Boston University School of Medicine, Director of the Evans Memorial Hospital, and Physician in Chief of the Massachusetts Memorial Hospital, will be a guest speaker. All physicians interested in allergy are welcome at the scientific sessions of the Academy meeting.

The meeting will be preceded by a postgraduate course in allergy to be held on Feb. 24 and 25, arranged by Dr. Francis C. Lowell, Associate Professor of Medicine and Assistant Dean of the Boston University School of Medicine. This course promises to be of interest to both the experienced allergist and the beginner in the field.

The Committee on Arrangements is featuring an enjoyable program of entertainment and the Ladies Committee has arranged for guided tours to historical landmarks, including Concord and Lexington, as well as teas and luncheons.

Complete programs of the meeting and postgraduate course are being mailed to all members and fellows of the Academy. Others who are interested may obtain them from the Executive Secretary, The American Academy of Allergy, 208 East Wisconsin Ave., Milwaukee 2, Wis.

Audio Visual Aids in Teaching Allergy

The Audio Visual Committee is anxious to obtain from members of the Academy any available data on slides and/or movies on the subject of allergy.

All information may be forwarded to:

Dr. Abraham Colmes, Chairman of the Committee,
416 Marlborough St.,
Boston 15, Mass.

New International Committee of the American Academy of Allergy

In order to improve relations between the American Academy of Allergy and allergists in other countries the International Committee has been enlarged to include representatives of a greater number of different countries. The new Committee is as follows:

Howard Osgood, M.D., Chairman, Buffalo, N. Y.
Horace S. Baldwin, M.D., New York, N. Y.
Egon Brunn, M.D., Copenhagen, Denmark
Stearns S. Bullen, M.D., Rochester, N. Y.
Gustavo Castaneda, M.D., Guatemala City, Guatemala
Jose L. Cortes, M.D., Mexico, D.F., Mexico
Carlos Cuervo-Trujillo, M.D., Bogotá, Colombia
Samuel M. Feinberg, M.D., Chicago, Ill.

Cesare Frugoni, M.D., Rome, Italy
Jose B. Greco, M.D., Belo Horizonte, Brazil
M. Coleman Harris, M.D., Mill Valley, Calif.
Carlos Jimenez-Diaz, M.D., Madrid, Spain
Willem, Kremer, M.D., Amsterdam, Holland
W. Loeffler, M.D., Zurich, Switzerland
A. M. Marchand, M.D., Santurce, Puerto Rico
Julio A. Moretti, M.D., Montevideo, Uruguay
Tell Nelson, M.D., Honolulu, Hawaii
José M. Quintero, M.D., Havana, Cuba
Francis M. Rackemann, M.D., Boston, Mass.
Ben Z. Rappaport, M.D., Chicago, Ill.
Bram Rose, M.D., Montreal, Canada
Arturo Rotor, M.D., Manila, Philippine Islands
Guido Ruiz-Moreno, M.D., Buenos Aires, Argentina
Ernst B. Salen, M.D., Stockholm, Sweden
Max Samter, M.D., Chicago, Ill.
Charles Sutherland, M.D., Melbourne, Australia
Pasteur, Vallergy-Radot, M.D., Paris, France
Amadeo V. Mastellari, M.D., Ancon, Canal Zone, Rep. de Panama
David A. Williams, M.D., Cardiff, Wales

Announcement

Meeting of the American College of Allergists

The Ninth Annual Congress of the American College of Allergists will be held at the Conrad Hilton Hotel, Chicago, April 27 to 29, 1953. In addition to general scientific sessions, there will be meetings of sections devoted to dermatology, pediatrics, psychosomatics, and otolaryngology. The final program will be announced later.

The meeting will be preceded by a Graduate Instructional Course in Allergy at the same hotel on April 24 to 26. All important phases of allergy will be covered in the general course and there will also be luncheon meetings on various special subjects.

Further information may be obtained from the Secretary-Treasurer, The American College of Allergists, 401 La Salle Medical Bldg., Minneapolis 2, Minn.

Correspondence

CHICAGO, ILL.
NOVEMBER 4, 1952

To the Editor:

In the September, 1951, issue of the JOURNAL, page 466, there was an article by Dr. Perkins and myself on Nickel and Cobalt Dermatitis. I have received several inquires concerning the nature of the cross-reactivity between the nickel and cobalt. It is true that most commercial nickel preparations include reasonable amounts of cobalt and vice versa, so that unless special care in taken, an idnividual when tested with a nickel compound will willy-nilly be also tested with cobalt and vice versa. The actual basis for the cross-sensitivity, however, is not the fortuitous inclusion of the one salt with the other, as tests with reasonably pure materials may give reactions to both elements. We, apparently, did not bring this out clearly enough in our article.

Since writing the article, through the courtesy of Dr. R. R. Suskind of the Kettering Laboratory of the College of Medicine of the University of Cincinnati, various of the actual compounds we employed were spectrographically examined to determine the concentrations of the other element in them. The results were:

Sample	*% Nickel*	*% Cobalt*
$Co(NO_3)_2 \cdot 6H_2O$	Nil or 0.05	
$(CoCl_5NH_2)Cl$	Nil or 0.05	
$Co(NH_3)_6Cl_3$	0.05	
$Co(C_2H_3O_2)_2 + 4H_2O$	0.05	
$Ni(NO_3)_2 \cdot 6H_2O$		0.1

In view of the fact that our test solutions were never stronger than 2 per cent, the concentration of nickel in our cobalt-testing solutions would then be in the order of 0.001 per cent or less, and this would appear to be an infinitesimal amount of contaminant. Commercially obtained material, even though supposedly pure, may not be so, as Dr. Suskind informed me that a commercial preparation of $CoCl_2 \cdot 6H_2O$ alleged to contain only 0.08 per cent of nickel was found, on spectrographic analysis, to contain at least 1 per cent of nickel.

A. ROSTENBERG, JR., M.D.

Book Reviews

Pharmacology in Clinical Practice. By Henry Beckman, M.D., Philadelphia, W. B. Saunders Company, 1952. 770 pages.

A Textbook of Pharmacology. By William T. Salter, M.D., Philadelphia, W. B. Saunders Company, 1952. 1201 pages.

Few branches of medical science have expanded so rapidly in recent years as pharmacology. Entirely new classes of valuable drugs such as antibiotics and antihistaminics have been discovered, and the availability of pituitary and adrenal hormones in adequate quantities has led to the discovery of therapeutic effects entirely unrelated to the old concept of replacement therapy. Simultaneously, increasing understanding of the relation of chemical structure to drug action has permitted development of many new synthetic compounds as modifications of, and substitutes for, the older drugs derived from botanical sources.

Such rapid progress makes it almost impossible for the practitioner to keep abreast of the literature and the natural tendency is to pick up information from the flood of advertisements that reach his desk and from the detail men of pharmaceutical manufacturers, both convenient but obviously biased sources. This situation increases the need for up-to-date critical textbooks but at the same time makes it increasingly difficult for an author to cover the subject adequately. The two present volumes will help the physician to evaluate the many new preparations that are available and use them intelligently in his practice. Even the allergist who has lost contact with general medicine should find them of value so that he will understand the medications that other physicians are prescribing for his patients and their possible toxic or allergenic effects.

The viewpoints of the two books are quite different. Beckman's new text, offered as a successor to his "Treatment in General Practice" which has been widely used for the past twenty years, is again essentially an exposition of therapy. The main portion of the book discusses practical therapeutics of various types of diseases, grouped according to medical specialties, with only the essentials of the underlying pharmacologic theory. The second section, an oversized appendix, lists alphabetically the drugs discussed in the first section. In conformity with the practical nature of the book, proprietary names are carefully cross-indexed and detailed information to manufacturers and dosage forms is included.

Salter's book presents a more formal approach to the science of pharmacology, with the major portion of the book devoted to Drug Actions on Physiological Mechanisms and shorter sections on The Application of Drugs in Clinical Medicine and on Toxicology. The discussions of chemistry, pharmacodynamics, and elimination of drugs from the body are far more complete than those of Beckman but the section devoted to clinical uses is less extensive. The arrangement of material in various sections is not altogether logical as one finds the discussion of respiratory poisons included in the section on applications to clinical medicine, rather than under Drug Actions or Toxicology.

Both books are written from the author's personal viewpoints with free expression of individual prejudices. This is particularly true of Beckman's book which makes few references to the literature, although a brief bibliography is appended to each chapter. Salter gives complete references but devotes little time to subjects in which he is not interested. For example, reference to his book for information on the uses and toxic effects of

gold salts yields little beyond the statement that they are an obsolete form of treatment not recommended by the author. Beckman, incidentally, does little to endear himself to the allergist, starting the first page of his book with the statement: "You will probably observe, as I have, that allergists are an apprehensive group. This is not surprising since their preferred immunologic approach is constantly being assailed by such brash fellows as psychiatrists, endocrinologists and pharmacologists. . . . The trouble with this prophylactic approach is that it does not work very well. . . . It is certain that without drugs he could not continue in practice as more than a mere diagnostician." This opinion, whether or not one accepts it as an essential part of the science of pharmacology, should offend only those who have failed to make use of all possible approaches to the treatment of allergic diseases.

Both books can be recommended for guidance in the use of the newer drugs. Beckman offers a simpler and more practical but somewhat superficial text for the general practitioner. Salter's is a more scholarly reference book with greater appeal to the more highly specialized physician.